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THE NATURE AND INTERACTION OF GENES DIFFERENTIATING HABIT OF GROWTH IN A CROSS BETWEEN VARIETIES OF TRITICUM VULGARE[1]

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INTRODUCTION

Although the multiple-factor hypothesis to explain the inheritance of quantitative characters was advanced in 1909 by Nilsson-Ehle (*25*)[3] and in 1910 by East (*6*), comparatively little is known concerning the nature and interaction of genes involved in the differentiation of quantitative characters. The study of the inheritance of quantitative characters in tobacco by Hayes (*15*) supported the hypothesis of Nilsson-Ehle and East and gave proof of the cumulative effect of the factors involved. Sax (*32*), Griffee (*14*), and Sirks (*33*) were the first to study factors for quantitative characters by means of their linkage relationship with factors for the production of qualitative characters. This method was used later by Immer (*17*) and by Lindstrom (*20, 21, 22, 23*), who has made a rather intensive study of the linkage between genes for the production of quantitative and those for the production of qualitative characters in the tomato. Lindstrom's studies furnish rather conclusive evidence that major factors for size of fruit occur in the tomato. Student (*1*), using a statistical mode of attack, estimates that at least 100 to 300 genes are necessary to explain the results of Winter's selection experiment for high oil content in maize. Fisher, Immer, and Tedin (*8*) developed a statistical method for studying the inheritance of quantitative characters. This method is particularly applicable to those cases in which the two parents differ by a large number of genes. The present paper reports the results of a study on the interaction and nature of genes differentiating habit of growth in crosses between varieties of wheat, *Triticum vulgare* Vill.

Considerable work has been done on the inheritance of growth habit in crosses between spring and winter varieties of *Triticum vulgare* and *T. compactum*. Spillman (*34*) obtained a 3 winter: 1 spring segregation in the F_2 generation of a cross between winter and spring wheats, indicating that the winter habit of growth is dominant to that for spring. Gaines (*9*), in crosses between two varieties of barley, and Cooper (*5*), in crosses between two varieties of wheat, obtained 13 spring forms: 3 winter forms in the F_2 generation. These results of

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[3] Reference is made by number (italic) to Literature Cited, p. 603.

Gaines and Cooper can be explained by assuming that the parents differed by two factor pairs, one being dominant for spring habit of growth and the other for winter habit with the dominant factor for spring habit of growth epistatic to the dominant factor for winter habit of growth. That some of the factors for winter habit of growth may be dominant to their allelomorphs is shown also by the preponderance of winter segregates in some F_3 families in a study reported by Hayes and Aamodt (*16*). Spillman (*34*) reported a single-factor pair involved in differentiating habit of growth, whereas Cooper (*5*) and Quisenberry (*27*) obtained results which indicated that the parents involved differed by two factor pairs. Vavilov and Kouznetsov (*38*), Aamodt (*2*), Gaines and Singleton (*11*), and Hayes and Aamodt (*16*) found that habit of growth was dependent upon multiple factors and did not attempt to determine the number of factor pairs involved.

MATERIAL AND METHODS

MATERIAL USED

The material used in this study consisted of 380 F_3 families from the cross Hybrid 128×Velvet Node, the F_2 generation of the cross Hybrid 128×Velvet Node, and the parents sown as checks. The F_3 generation was sown on only one date, April 4. The seed from one head of each of the 380 F_2 plants was sown in rows 1 foot apart, the seed within each row being spaced 3 inches apart. For the F_2 generation, the seed of the F_1 cross was divided into three equal parts. This made possible three dates of planting: February 28, April 4, and May 4. The seed of these crosses was spaced 6 inches apart within the rows, and therefore is not comparable with the F_3 generation data, as the seeds producing the F_3 plants were spaced only 3 inches apart. Under conditions of limited rainfall, such as prevail at Pullman, Wash., where the study was conducted, the greater spacing of the seed giving rise to the F_2 generation would cause the plants of this generation to mature later than those of the F_3. Seed of the F_2 generation of Hybrid 128× Velvet Node giving rise to the F_3 was sown in the autumn of 1923. Bluestem, a spring wheat, came through the winter of 1923–24 with a stand of 92 percent, whereas Hybrid 128 in the spring had a stand of 96 percent. This showed that no material winter-killing of fall-sown spring wheat had occurred.

The date of ripening was chosen as the means of separating the different progenies. A plant was considered ripe and was harvested when the outer glumes and the visible part of the inner glumes as well as the base of the rachis of the tallest culm had turned yellow.

The harvesting of the plants on the basis described above was divided into 10 weekly periods. The first weekly period began July 21. Following this date the ripened plants were pulled at the end of each week and the tallest culm of each plant collected for further study. The last or eighth period of harvesting was September 14. On this date all plants were pulled and divided into three groups—those ripe, those headed but not ripe, and those not headed. In making these divisions a plant was considered as headed if one spike had its lowest spikelet fully emerged from the uppermost leaf sheath. In analyzing the data those plants headed but not ripe and those not headed were considered as belonging to the ninth and tenth periods, respectively.

STATISTICAL METHODS EMPLOYED

To group those families in the F_3 generation having like segregation and to test whether the segregation fitted a given hypothesis, a method based on that devised by Emerson [4] for testing Mendelian ratios was employed. Probability tables for Mendelian ratios with small numbers have recently been published by Warwick (*39*).

For grouping families of like segregation, limits of 1.96 times the standard deviation of the binomial distribution were employed. Such a test gives odds of 19 : 1 against deviations in excess of 1.96 times the standard deviation being due to the errors of random sampling, and may be used, therefore, to set up limits of sufficient magnitude that deviations in the number of families expected in any one group of the data shall occur but infrequently.

This method of determining the limits of segregation applies to the group as a whole and does not take into consideration the variability in numbers of plants in F_3 families. For that reason the few F_3 families having less than 20 plants per family were discarded. By so doing it was not found necessary to apply the analysis to each F_3 family, but instead they could be treated as a group by using for n in the formula for the standard deviation of the binomial distribution the average number of plants in all the families. For those F_3 families having some winter segregates the average number of plants per family was 42 and for those not having any winter segregates the average frequency was 39.

To test whether the observed data differed from the theoretical expected by a margin greater than can be explained by the errors of random sampling, the χ^2 test for goodness of fit was employed. The test was applied to both the distributions of the F_3 families themselves and the total distribution within F_3 families grouped on the basis of their segregation. The correctness of the grouping could be tested further by applying the χ^2 test to each F_3 family individually, as outlined by Fisher (*7, ch. IV*). This would give a test as to whether the F_3 families themselves show any sign of departure from the segregations expected. Since, in many cases the number in some of the observational categories is less than 10, the application of such a test to the data considered in this article is questionable. This is pointed out here since other workers may have sufficiently large numbers to make such an analysis desirable. Genes other than those differentiating habit of growth are involved in the cross also. The number of such factor pairs of major importance is probably not more than 1 or 2, as is indicated by the fact that their presence is discernible in practically every table. The influence of such factors would be expected to prohibit the F_3 families from F_2 plants of a certain known genotype from following the normal distribution.

ADJUSTMENTS TO COMPENSATE FOR OVERLAPPING OF CLASSES AND SMALL NUMBER OF PLANTS IN F_3 FAMILIES

A provisional hypothesis based on a preliminary study of the 380 F_3 lines greatly facilitates the analysis and for that reason is given here even though the proof or disproof of the correctness of such a hypothesis must come from the data. The three factor pairs differentiating the spring habit of growth possessed by Velvet Node from the winter

[4] EMERSON, R. A. Mimeographed tables., Cornell Univ., Dept. Plant Breeding

habit of growth possessed by Hybrid 128 were designated as *Aa*, *Bb*, and *Cc*. *AA*, *BB*, and *cc* were found to be factors for spring habit of growth and *aa*, *bb*, and *CC* factors for winter habit of growth, with *AA* being epistatic to *bb* and *CC*, *BB* being partially epistatic to *aa* and *CC* and *cc* being partially epistatic to *aa* and *bb*. The great majority of the plants of the genotype *AA*— ripened in class I, which includes period 1; plants of the genotype *aaBBcc* ripened in class II, which includes periods 2, 3, and 4; plants of the genotype *aaBBCC* or *aabbcc* had the great majority of their plants ripening in class III, which includes periods 5, 6, 7, 8, and 9; and the great majority of the plants of the genotype *aabbCC* fell in class IV, which includes period 10.

As has been pointed out, the plants of each F_3 family were classified by weekly periods. It would be surprising indeed if the distribution of plants of some genotypes did not overlap, making it impossible to set up a classification based on the effect of genotypes such that plants of some factorial constitutions did not fall into more than one period. This is especially true because of the segregation of factors for earliness other than those being studied, which differentiate habit of growth. In order to be able to test the observed distribution against a theoretical expected on the basis of a specified genotype, it is necessary to make certain adjustments. This can be accomplished by adjusting the observed frequency on the basis of the theoretical or by adjusting the theoretical. For the sake of clarity the former method has been used in this analysis. It seems desirable at this time to discuss the adjustments made.

The data show that 2.6 percent of plants of the genotype *AA*—, and of those *Aa*— genotypes ripening plants predominantly in class I, fall in class II. To correct for this overlapping, 2.6 percent of the theoretical number of plants expected in class I was subtracted from the number observed in class II and added to the number observed in class I. This adjustment was applied uniformly to all groups having segregates in class I.

Another adjustment was that for the interaction of *Cc* in the genotype *aabbCc* such that 26 percent of the plants of this genotype ripened in class III and 74 percent in class IV. In this case 26 percent of the number of plants of this genotype expected was subtracted from class III and added to class IV. This correction is applied to the data for all groups of F_3 families whose distributions are being tested to determine whether they fit the theoretical based on the expected behavior of a genotype involving *aabbCc*.

Among those F_3 families segregating winter plants would be found some derived from F_2 plants of the genotypes *AaBbCc* and *AaBbCC*. Of the plants from these F_3 families, 3.875 and 6.25 percent, respectively, would be expected to ripen in class IV, which is based on the behavior of the winter parent, hybrid 128. Since the average number of plants in the F_3 families having some winter segregates is 42, the F_3 families of the genotypes *AaBbCc* and *AaBbCC* would be expected to fluctuate around means of 1.6 and 2.6 plants in class IV. It is evident that owing to errors of random sampling some of the F_3 families of these genotypes would have no segregates in the fourth period, and since the presence or absence of plants in period IV is used as a basis for separating those F_3 families of the genotypes expected to produce winter segregates from those homozygous for at least one factor pair for spring habit of growth, it is evident that some

F_3 families have been incorrectly classified. The expected number and segregation within such families can be predicted by the use of the formula for the binomial distribution and adjustments made.

These corrections are important in testing the distribution of plants within F_3 families, and therefore have been applied to all the total distributions of those F_3 families to which such an adjustment should be applicable. The results are given after "Obtained" in column 2 of table 12. Since in the analysis of the data which follow, these adjustments are made in getting the obtained to test against the theoretical, the reader desiring to check the calculations must keep them in mind.

EXPERIMENTAL RESULTS

The experimental results may be logically analyzed under two parts, namely, the F_3 generation data and the effect of the environmental conditions following different dates of planting upon the reaction of factors differentiating habit of growth.

SEPARATION OF F_3 FAMILIES INTO GROUPS ACCORDING TO GENOTYPE OF F_2 PLANTS GIVING RISE TO THEM

As regards the F_3 generation data, the nature of the action and interactions of the factors differentiating habit of growth in the cross between Hybrid 128 × Velvet Node must be determined by the behavior of F_3 families derived from F_2 plants of the same genotype. To derive this information it is necessary to separate the 380 F_3 families into groups according to the genotype of the F_2 plants giving rise to them. Since, as will be shown later, the Velvet Node and Hybrid 128 parent are differentiated by three factor pairs, a total of 27 groups would be expected if complete separation were possible. After the separations have been made their validity must be tested. Therefore, the analysis of the F_3 generation must provide a grouping of the F_3 families according to the genotypes of their F_2 parents, and must test the validity of this grouping.

The behavior of the parents is used as a measure for determining the genotype of the F_2 plants giving rise to specified F_3 families. Velvet Node ripened all of its plants during the first period, whereas Hybrid 128 had 2 percent of its plants falling in the ninth period and 98 percent in the tenth period.

F_3 Families with Plants in Either the Ninth or Tenth Period or Both

The F_3 families with plants in either the ninth or tenth period only or in both are listed in table 1. The distribution of the plants in these F_3 families is similar to that for the Hybrid 128 parent, and therefore these F_3 families are considered as the progeny from F_2 plants homozygous for the factors determining winter growth habit. The number of such F_3 families is 6, which very closely approximates the 5.94 expected out of 380 on the assumption that the parents are differentiated by three main factor pairs. If three main factor pairs do differentiate winter and spring habit of growth in this cross, the expected ratio of F_3 families having no winter plants to those having some winter plants is 37:27, or, on the basis of 380 F_3 families, 219.69: 160.31. The obtained ratio is 221: 159, which is convincing evidence that spring habit of growth in the cross Hybrid 128 × Velvet Node is differen-

tiated from winter habit of growth by three main factor pairs. The complete analysis of the data supports this conclusion, but that point is not emphasized since the primary interest lies in the nature of the action and the interaction of the genes. The F_3 families listed in table 1 are considered to have been derived from F_2 plants of the genotype *aabbCC*.

TABLE 1.—*F_3 families with plants in either the ninth or tenth period, or both, F_2 genotype aabbCC*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Segregates in tenth period
	I	II			III					IV		
	1	2	3	4	5	6	7	8	9[1]	10[1]		
									Number	*Number*	*Number*	*Percent*
1431										37	37	100.00
1595										29	29	100.00
1689										64	64	100.00
1502									1	45	46	97.8
1440									1	38	39	97.4
1399									1	31	32	96.9
Total									3	244	247	

[1] Period 9 includes plants headed but not ripe on Sept. 14, and period 10 includes plants not headed on that date.

F_3 Families Having Segregates in the First or First and Second Periods Only

F_3 families having segregates in the first and second periods only are given in table 2. In addition to these 7 F_3 families, there are 75 whose behavior was identical with that of the Velvet Node parent, which, it will be remembered, ripened all its plants in the first period. This makes a total of 82 F_3 families ripening all but 2.6 percent of their plants in class I. On the assumption that a dominant factor pair in the homozygous condition is capable of ripening all but 2.6 percent of the plants in class I, 95 such F_3 families would be expected among the 380 included in this study. The χ^2 test for goodness of fit between the expected and obtained gives a P value between 0.20 and 0.10, and therefore the deviation of 13 may be attributed to the errors of random sampling. Other assumptions, such as the postulation that any two spring factors alone would cause plants to ripen the first period, were tried, but since none was found which would fit the F_3 data in its entirety and as the one advanced does, it seems safe to conclude that one of the dominant spring factor pairs causes all but 2.6 percent of the plants to ripen in class I. The factor pair having such an effect is designated *AA* and it is evident that *AA* must be epistatic to the *bb* and *CC* factor pairs for the determination of winter habit of growth. Then, the plants recorded in table 2 must have been derived from F_2 plants of the genotype *AA*—.

It is obvious from an examination of table 2 that all the differences in the percentage of plants ripening during the first period cannot be due to the errors of random sampling. This is particularly true of F_3 families 1637 and 1413, in which only 56.4 and 20.4 percent of the plants, respectively, ripened during the first period, and therefore

the distribution within these two F_3 families as compared with that of the families ripening all of their progeny in period 1 is evidence of the presence of factors for earliness other than those differentiating winter from spring habit of growth in the cross Hybrid 128 × Velvet Node.

TABLE 2.—*F_3 families having segregates in the first and second periods only, F_2 genotype AA*—

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Plants ripening the first period
	I	II			III					IV		
	1	2	3	4	5	6	7	8	9	10		
											Number	*Percent*
1601	38	1									39	97.4
1364	30	2									32	93.8
1376	29	3									32	90.6
1669	44	8									52	84.6
1602	38	7									45	84.4
1637	31	24									55	56.4
1413	11	43									54	20.4
Total	221	88									309	

F_3 FAMILIES HAVING NO SEGREGATES EARLIER THAN THE FIFTH PERIOD

F_3 familes from F_2 plants heterozygous for one factor pair in which pring habit of growth is dominant, would fluctuate around a mean of 25 percent winter segregates, and this would be the largest mean percentage of winter segregates possible on the assumption that all factors for spring habit of growth are dominant to their allelomorphs. Due to the errors of random sampling, the chances against F_3 families occurring with more than 38.1 percent of winter segregates is 39.1, as determined by the application of the formula for the standard deviation of the binomial distribution. Then the 14 F_3 families listed in table 3 which range from 41.2 to 77.6 percent of plants in the tenth period, which includes only winter segregates, can be accounted for on the basis that one of the factors for winter habit of growth is at least partially dominant over its allelomorph for spring habit of growth. The factor pair having such a reaction is designated *CC*. Then the genotype of the F_2 plants giving rise to the F_3 families listed in table 3 must have been *aabbCc*.

The validity of the assumption that table 3 includes those F_3 families from F_2 plants of the genotype *aabbCc* can be tested by comparing the number obtained with the number expected. In this cross 11.88 of the total 380 F_3 families would be expected to be from F_2 plants of this genotype. This is very close to 14, the number obtained.

A knowledge of the reaction of plants having the genotype *aabbCc* is essential to a correct separation of those F_3 families in which this genotype appears. Since table 3 gives considerable information on this point, it seems desirable at this time to determine the effect of the *aabbCc* genotype. The distribution into classes III and IV of the segregates from F_2 plants of the genotype *aabbCc* is dependent upon the degree to which this genotype is able to approach the reaction of

the genotype *aabbCC* of the winter parent, which, it will be remembered had all but 2 percent of its plants falling in class IV. The influence of the *aabbCc* genotype upon maturity can be predicted from the behavior of F_3 families from F_2 plants of the genotypes *aabbCc*, *aaBbCc*, *AaBbCc*, and *AabbCc*, as the genotype *aabbCc* will appear among the progeny of all such F_2 plants. F_3 families of the genotype *aabbCc* are listed in table 3 and the total distribution for those of the genotype *aaBbCc* in table 9, and those of the genotypes *AaBbCc*, *AabbCc*, and *aaBbCc* in table 8. From a preliminary examination of these tables it was determined that approximately 26 percent of the plants of the genotype *aabbCc* fell in period 9 of class III and the remaining 74 percent in class IV. If this assumption is correct the total distributions listed in tables 3, 8, and 9 must fit the theoretical expected after being adjusted for the 26 percent of the *aabbCc* genotypic plants that fall in class III which, if dominance were complete, as is assumed in calculating the theoretical expected, would, with the exception of 2 percent, fall in class IV.

TABLE 3.—*F_3 families having segregates within the fifth to the ninth period and more than 38.1 percent of the segregates in the tenth period, F_2 genotype aabbCc*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Segregates in tenth period
	I	II			III					IV		
	1	2	3	4	5	6	7	8	9[1]	10[1]		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1492							10		1	38	49	77.6
1539					1	4	3		1	31	40	77.5
1618					6	2	3			37	48	77.1
1433					6	1				21	28	75.0
1685						8	5			35	48	72.9
1565						2	2	1	3	21	29	72.4
1466							5	2	7	28	42	66.7
1316					1	3			3	14	21	66.7
1369					4	3			6	26	39	66.7
1617					9	5	2		1	28	45	62.2
1696						11	7		7	35	60	58.3
1549					1	4	8		7	26	46	56.5
1310					4	5	2	1	11	23	46	50.0
1420						1	12	1	16	21	51	41.2
Total					32	49	59	5	63	384	592	

[1] Period 9 includes plants headed but not ripe on Sept. 14, and period 10 includes plants not headed on that date.

The adjusted total distribution for table 3 is 131 : 461, which tested by χ^2 for goodness of fit to the theoretical ratio of 148 : 444 gives a P value between 0.20 and 0.10. The deviations noted may be accounted for by the errors of random sampling. Table 3 supports the supposition that *C* is only partially dominant to *c*.

Table 3 shows that none of the F_3 families from F_2 plants of the genotype *aabbCc* ripened plants earlier than class III. The remainder of those F_3 families having some winter segregates but not ripening any plants earlier than class III are listed in table 4. The F_3 families in this table appear to be varying around a mean of 25 percent of plants in period 10, or winter segregates. If such is the case the total distribution given at the bottom of table 4 should approximate a ratio of 3 plants in class III: 1 plant in class IV, which is expected on the

assumption that the F_2 plants giving rise to these F_3 families are heterozygous for one factor pair dominant for spring habit of growth. On this basis the expected ratio from a total population of 402 is 301.5: 100.5, and the obtained ratio is 316 : 86. The *P* value obtained by applying the χ^2 test for goodness of fit does fall just below 0.10, and therefore the deviations are not considered as statistically significant. If the F_3 families listed in table 4 are the descendants of plants heterozygous for one factor pair, the number expected from 380 families is 11.88, which agrees very well with the 10 obtained. The data show rather conclusively that the F_2 parents of the F_3 families listed in table 4 were heterozygous for a dominant spring factor pair.

TABLE 4.—F_3 *families having segregates within the fifth to the ninth period and less than 38.1 percent of the segregates in the tenth period, F_2 genotype aaBbCC*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Segregates in tenth period
	I	II			III					IV		
	1	2	3	4	5	6	7	8	9[1]	10[1]		
					Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1496					1		6	3	7	10	27	37.0
1472					2	1	10	5	14	14	46	30.4
1454					1			4	27	12	44	27.3
1467					1		6	2	33	15	57	26.3
1533					2	1	5	5	25	13	51	25.5
1363						3	11	2	16	8	40	20.0
1398						4	11	2	13	5	35	14.3
1653					2	6	12	7	8	5	40	12.5
1592					1	3	12	4	7	2	29	6.9
1378					7	10	11		3	2	33	6.1
Total					17	28	84	34	153	86	402	

[1] Period 9 includes plants headed but not ripe on Sept. 14, and period 10 includes plants not headed on that date.

From the 27 genotypes expected if growth habit is differentiated by three factor pairs, *AabbCC* and *aaBbCC* are the only ones heterozygous for one dominant spring factor pair which would give rise to progeny segregating for spring and winter habit of growth. Since some of the progeny from F_2 plants of the *AabbCC* genotype would be expected to ripen in class I and as none of the plants listed in table 4 ripen earlier than class III, the F_3 families listed in this table must have arisen from F_2 parents of the genotype *aaBbCC*.

Among the segregates from the F_2 plants of the *aabbCc* genotype would be plants of the genotype *aabbcc*, and among the progeny of F_2 parents of the *aaBbCC* genotype would be segregates of the genotype *aaBBCC*. Since the earliest that any of the F_3 families in either table 3 or 4 ripened plants was class III, the great majority of the progeny from the F_2 plants of the genotype *aabbcc* or *aaBBCC* would be expected to fall in class III. The F_3 families having all of their progeny ripening in class III are tabulated in table 5. The number of families of these two genotypes expected among 380 is 11.88, which approximates the number obtained, 9. That some F_3 families from F_2 plants of the genotypes *aabbcc* and *aaBBCC* occasionally ripen a few of their progeny in period 4 of class II seems evident from an analysis of the total distribution of the progeny of *aaBbcc* and *aaBBCc* given in table 8. This matter is taken up in detail later, but

it seems appropriate at this time to point out that since plants of the genotype *aabbcc* or *aaBBCC* are expected in only one-fourth of the progeny from F_2 plants of the genotype *aabbCc* or *aaBbCC*, respectively, F_3 families from F_2 plants of these latter two genotypes would rarely be expected to have plants maturing earlier than the fifth period, as compared to F_3 families from F_2 plants of the genotype *aabbcc* or *aaBBCC*, in which 100 percent of the progeny would be of the genotype *aabbcc* or *aaBBCC*, respectively. Therefore, the method used in isolating the F_3 families derived from F_2 plants of the genotypes *aabbCc* and *aaBbCC*, listed in tables 3 and 4, respectively, is believed to be sound.

TABLE 5.—*F_3 families having all plants within the fifth to the ninth period, inclusive, F_2 genotypes aabbcc and aaBBCC*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants
	I	II			III					IV	
	1	2	3	4	5	6	7	8	9[1]	10[1]	
					Number	*Number*	*Number*	*Number*	*Number*		*Number*
1650					4	17	11	5			37
1547					3	16	10	3	3		35
1442					8	11	13	3	5		40
1490					6	9	12	1	5		33
1608					5	13	15	1	7		41
1572						8	29	2	6		45
1421						9	11	6	10		36
1469					2	7	5	4	10		28
1536						3	7	5	22		37
Total					28	93	113	30	68		332

[1] Period 9 includes plants headed but not ripe on Sept. 14, and period 10 includes plants not headed on that date.

F_3 FAMILIES RIPENING ALL THEIR PLANTS IN CLASS II

There were six F_3 families which ripened all of their plants within the second, third, and fourth periods (table 6). Since it has been shown that *AA* — ripens all but 2.6 percent of its plants in the first period, or in class I, and that *aabbcc* and *aaBBCC* ripen all but a few of their plants in class III, it seems logical to conclude that these six families represented the cumulative effects of *BB* and *cc* in combination with *aa* and therefore were from F_2 plants of the genotype *aaBBcc*. Among the total of 380 F_3 families, 5.94 such F_3 families would be expected, which number is very close to the 6 obtained.

TABLE 6.—*F_3 families having all plants within the second to the fourth period, inclusive, F_2 genotype aaBBcc*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants
	I	II			III					IV	
	1	2	3	4	5	6	7	8	9	10	
		Number	*Number*	*Number*							*Number*
1358		24									24
1471		24									24
1473		24									24
1503		3	47								50
1639		6	35								41
1543				40							40
Total		81	82	40							203

Since these 6 families are scattered over 3 weekly periods of ripening, 3 ripening in the second period and 1 in the fourth, it is evident, as previously postulated, that factors other than the ones being studied are operating to produce earliness and lateness.

F_3 Families Having Plants in Class I and Either in Class II or III, or Both

The F_3 families listed in table 7 have segregates in the first period, and within the second to the fourth period, inclusive. Since *AA*— is known to ripen plants the first period and *aaBBcc* the second, third, or fourth period, this is the distribution expected from F_2 generation plants of the genotype *AaBBcc*. The number of such families expected from a total of 380 is 11.88 and the number obtained is 14. It seems safe to conclude that these F_3 families are from F_2 plants of the genotype *AaBBcc*. Since the effect of the *AaBBcc* genotype among the progeny from *AaBBcc* plants is the only unknown, it should be possible to derive this information from the distribution within F_3 families of table 7. The obtained ratio corrected for the 2.6 percent overlapping of classes I and II is 371 in period 1:138 within periods 2 to 4, inclusive. The expected on the basis of a 3:1 segregation is 381.75:127.25. The χ^2 test for goodness of fit gives a *P* value between 0.30 and 0.20. It seems that *AaBBcc* causes plants to ripen the first period. The data in table 7 also confirm the conclusions previously reached as to the effect of the *AA*—genotype.

TABLE 7.—*F_3 families having segregates in the first period and within the second to the fourth period, inclusive, F_2 genotype AaBBcc*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Plants ripening the first period
	I	II			III					IV		
	1	2	3	4	5	6	7	8	9	10		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1422	36		1								37	97.3
1389	33		2								35	94.3
1468	47			3							50	94.0
1583	23		4	1							28	82.1
1598	23		2	3							28	82.1
1650	21			5							26	80.8
1401	25	2	4	1							32	78.1
1308	24	3	1	3							31	77.4
1439	36	2	4	6							48	75.0
1321	19	2	5	1							27	70.4
1623	44	4	19								67	65.7
1476	13	15	2								30	43.3
1320	11	8		7							26	42.3
1657	6	29		9							44	13.6
Total	361	65	44	39							509	

Since the separation of the remaining F_3 families according to the genotype of their F_2 parents is dependent upon a thorough understanding of the effect upon maturity of the F_2 genotypes, already isolated, it seems desirable at this point to briefly summarize the findings. The genotype *aabbCC* was found to cause plants to fall in periods 9 and 10, with all but 1.2 percent in the latter class; *AA*— caused plants to ripen in periods 1 and 2, all but 2.6 percent falling in

the first period; *aabbCc* caused plants to fall in periods 9 and 10 in the proportion of 26:74; *aabbcc*, *aaBBCC*, and *aaBbCC* caused plants to ripen in periods 5, 6, 7, 8, and 9, or class III; *aaBBcc* caused plants to ripen in periods 2, 3, and 4, or class II; and *AaBBcc* caused plants to ripen in periods 1 and 2 in the proportion 97.4:2.6.

Since the above separations of F_3 families *delimits* the four different classes and since they (especially the data in table 6) demonstrate the presence of factors affecting earliness other than *Aa*, *Bb*, *Cc*, the segregations for individual F_3 families have been listed. For the remaining separations only the total distributions are given. These, together with the foregoing total distributions, are compiled in table 8. The total distributions are not listed in the order in which it is most logical to discuss them but are grouped in such a way as to facilitate comparisons between the different genotypes.

TABLE 8.—*Total distributions for the F_3 families descended from F_2 plants of different genotypes*

F_2 genotypes	Plants in weekly ripening periods dating from July 21, grouped in class—										Total F_3 families
	I	II			III					IV	
	1	2	3	4	5	6	7	8	9	10	
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
AA—	3,322	88									82
AaBBcc	361	65	44	39							14
AaBbcc	889	8	73	132	62	17	28	4	10		31
AaBBCc	642	215	100	130	49	25	21	7	16		32
Aabbcc	159	11			18	18	7	3	2		6
AaBBCC	129	131	157	34	36	34	44	4	27		15
aaBBcc		81	82	40							6
aaBbcc, *aaBBCc*		62	127	326	251	118	103	25	76		30
aaBBCC, *aabbcc*					28	93	113	30	68		9
AaBbCc, *AaBbCC*, *AabbCc*	1,425	518	654	296	122	112	113	43	178	280	87
AabbCC	65	54	87	18					7	57	8
aaBbCc		54	87	242	216	144	86	46	130	165	30
aaBbCC					17	28	84	34	153	86	10
aabbCc					32	49	59	5	63	384	14
aabbCC									3	244	6

From the foregoing analysis it was found that plants of the genotypes *AA*— and *AaBBcc* ripened in class 1 and that plants of the genotypes *aaBBCC* and *aabbcc* ripened predominantly in class III. Then segregates from F_2 plants of the genotypes *AaBbcc*, *AaBBCc*, *Aabbcc*, and *AaBBCC* should ripen within the classes I to III, inclusive. If plants of the genotypes *Aabbcc* and *AaBBCC* ripen in the first class as do plants of the genotype *AaBBcc*, then all F_3 families from F_2 plants of these genotypes should be fluctuating around a mean of 75 percent of the plants in the first period. Such was not found to be the case, as some F_3 families appeared to be fluctuating around a mean of 25 percent of plants ripening the first period. This would indicate that probably either the genotype *Aabbcc* or the genotype *AaBBCC*, or both, caused plants to ripen in class II, and therefore that these F_3 families were segregating in the ratio of 1:2:1 as regards classes I, II, and III.

To test this conclusion, the F_3 families having less than 38.6 percent of the plants in the first period, more than 11.4 percent of them after the fourth period, and none in the tenth period were grouped

together, and the total distributions are listed opposite *AaBBCC* in table 8. Adjusting for the 2.6 percent of the *AA*— genotype ripening in period 2 and the one F_3 family from an F_2 plant of the genotype *AaBbCC* which would be expected to have no winter segregates, the obtained is 123:297:134 and the expected is 138.5:277:138.5. The χ^2 test for goodness of fit give a P value between 0.20 and 0.10. These F_3 families may be considered as segregating in the ratio of 1 plant in class I : 2 plants in class II : 1 plant in class III.

The number of such families obtained after subtracting the expected 1 from an F_2 plant of the genotype *AaBbCC* is 14. The expected if both the *AaBBCC* and *Aabbcc* genotypes are present is 23.75. By applying the χ^2 test for goodness of fit, the P value falls between 0.05 and 0.02. The expected if only one genotype is present is 11.88. By applying the χ^2 test a P value between 0.50 and 0.30 is obtained. The data indicate that either *AaBBCC* or *Aabbcc* gives rise to the 14 families in table 8. The conclusive proof lies in the fact that the data from the entire 380 F_3 families will not fit the hypothesis that the segregation from these two genotypes is identical. The reader can substantiate this statement by attempting to fit this hypothesis to the data in the tables. As a working hypothesis, the F_3 families whose total distribution is listed in table 8 opposite *AaBBCC* are considered as having arisen from F_2 plants of the genotype *AaBBCC*, and that *Aabbcc* causes plants to ripen in the first period. Regardless of which genotype is present the data show that *Aa* in certain combinations with the other factor pairs is not capable of ripening plants the first period as does the homozygous dominant *AA*.

In the preceding discussion it was postulated that *Aabbcc* causes plants to mature at the same time as *AA*—, which has been shown to ripen all but 2.6 percent of its plants in class I. It has been shown previously that all but a very few of the plants of the genotype *aabbcc* fall in class III. Then F_3 families from F_2 plants of the genotype *Aabbcc* should have their plants distributed predominantly in classes I and III, with occasionally some plants in period 2 of class II. The total distribution for the F_3 families answering this description is listed opposite *Aabbcc* in table 8. From the previous data showing that *AA*— and *Aabbcc* ripen some plants in period 2, it is clear that the plants in period 2 are of these genotypes and therefore should be grouped with those in period 1. On this basis the obtained number of plants in class I is 170 and in class III is 48, whereas the expected if these F_3 families are derived from *Aabbcc* F_2 plants is 163.5:54.5. Further statistical analyses are not necessary to show that the fit is good. The number of F_3 families from a total of 380 expected to be of the genotype *Aabbcc* is 11.88 and the number listed in table 8 is 6. The χ^2 test for goodness of fit gives a P value between the 10- and 5-percent points. It seems safe, according to the working hypothesis, to conclude that the F_3 families listed in table 8 opposite *Aabbcc* are the progeny of F_2 plants having the genotype *Aabbcc*.

There remain 60 F_3 families having segregates within classes I, II, and III. This is the distribution expected of the progeny from F_2 plants of the genotypes *AaBBCc* and *AaBbcc*. For the purpose of determining the behavior of the factorial combination *aaBBCc* and *aaBbcc* it is desirable to separate the F_3 families derived from these F_2 genotypes.

According to the working hypothesis that the genotype *AaBBCC* ripened plants in class II, the maximum expected percentage of progeny from plants of the genotype *AaBBCc* ripening the first period would be 62.5. In order to arrive at this figure it must be remembered that plants with *aa* in their genotype ripen later than the first period. The other genotype, *AaBbcc* would be expected to have 75 percent of its progeny ripening during the first period. The maximum expected percentage of progeny from plants of the genotype *AaBBCc* ripening during the first period due to random sampling is 77 percent if $(1.96 \times \text{S.D.}) + 62.5$ is taken as the upper limit. The odds against plus deviations as great as this occurring as a result of random sampling are 39:1. Only one F_3 family exceeding 77 percent of plants ripening in the first period had two plants ripening in the second period, and only one had one plant ripening in the second period. Since these F_3 families should be from plants of the genotype *AaBbcc*, it was decided to try to separate the *AaBbcc* and *AaBBCc* genotypes on the basis of the number of plants ripening in the second period. All those F_3 families having one or no plants ripening in the second period were considered as being from F_2 plants of the genotypes *AaBbcc*. The total distribution of such F_3 families is listed opposite *AaBbcc* in table 8.

Whether this assumption is correct can be determined by testing the number of such families against the expected among a total of 380. The number of such families expected is 23.75 and the obtained was 31. The χ^2 test for goodness of fit gives a P value between 0.20 and 0.10. The data fit the hypothesis that the F_3 families whose total distributions are listed opposite *AaBbcc* in table 8 descended from F_2 plants of the genotype *AaBbcc*.

Since the reaction of all the genotypes of the progeny from F_2 plants of the genotype *AaBbcc* is known except *aaBbcc*, table 8 offers an opportunity to determine the behavior of plants possessing this latter genotype. As regards these families, plants having *AA* or *Aa* in their genotype have been shown to ripen in class I, those possessing *aaBBcc* in class II, and those possessing *aabbcc* in class III. The obtained number is 889:213:121. Because of the method of selecting these families it is not necessary to correct for the 2.6 percent of the *AA* and *Aa* genotypes that would be expected to ripen in period 2. The expected on the assumption that *aaBbcc* ripens plants in classes II and III in the proportion of 3:1 is 917.25:191.09:114.66. The assumption that *aaBbcc* ripens plants in classes II and III in the ratio of 1:1 will not fit the data, as the odds against the occurrence of deviations as great as would exist being due to the errors of random sampling are enormous. It is evident that the preponderance of plants of the genotype *aaBbcc* ripen in class II.

The remaining F_3 families ripening plants in classes I, II, and III only must be of the genotype *AaBBCc*. The total distributions for these F_3 families are listed opposite *AaBBCc* in table 8. The expected number of such families is 23.75. The number listed in table 8 is 32. However, under the discussion of methods it was stated that five F_3 families of the genotype *AaBbCc* would be expected to have no winter segregates due to the errors of random sampling, and therefore would have been classed with the progeny from F_2 plants of the genotype *AaBBCc*. Adjusting for these five families, the obtained is 27, which

is very close to the expected. It appears that with the possible exception of five families, the F_3 families opposite *AaBBCc* in table 8 probably descended from F_2 plants of the genotype *AaBBCc*.

The effect upon earliness is unknown for only 2 of the 9 genotypes segregating from *AaBBCc* plants. These two genotypes are *AaBBCc* and *aaBBCc*. From the data in table 8 it should be possible to determine the effect of *AaBBCc* and *aaBBCc* provided allowance is made for the five families previously noted. After making the necessary adjustments the proportion obtained from *AaBBCc* plants is 555:361:79. The expected on the assumption that the *AaBBCc* genotype ripens plants in the classes I and II in the ratio of 3:1 and that *aaBBCc* ripens plants in classes II and III in the ratio of 3:1, is 559.69:342.03:93.28. The *P* value calculated from the χ^2 test for goodness of fit lies between 0.20 and 0.10. The data show that *AaBBCc* as regards earliness is intermediate in its reaction between *AaBBcc* and *AaBBCC*, since *AaBBCC* was found to cause plants to ripen in class II and *AaBBcc* in class I. However, the effect of the *AaBBCc* genotype more nearly approaches that of the *AaBBcc* genotype. Likewise the *aaBBCc* genotype more nearly approaches the *aaBBcc* genotype in behavior than it does the *aaBBCC* genotype.

It can be shown that these data would fit the hypothesis that *AaBBCc* ripens plants in classes I and II in the ratio of 1:1, but such a hypothesis would not fit the data opposite *AaBbCc*, *AaBbCC*, *AabbCc* in table 8 in which this genotype appears again.

F_3 Families with Plants in Classes II and III

Since the genotype *aaBBcc* was found to cause plants to ripen in class II and *aaBBCC* and *aabbcc* were found to cause plants to ripen in class III, it would be expected that F_3 families derived from F_2 plants of the genotype *aaBbcc* and *aaBBCc* would cause plants to ripen in classes II and III. The total distributions for the F_3 families ripening plants in classes II and III are listed in table 8 opposite *aaBbcc*, *aaBBCc*. The number of such F_3 families expected is 23.75 and the obtained is 30.

The proportions of segregates in classes II and III can be predicted from the previous data. The expected proportion of plants in class II and III is 680:408 and the obtained is 515:573. Statistical methods are not necessary to show that the deviations are greater than can be explained by the errors of random sampling. Class III has a preponderance of segregates, which is exactly what would be expected if F_2 plants of the genotypes *aaBBCC* and *aabbcc* ripened a few of their plants in the fourth period, for F_3 families derived from such F_2 plants would have been grouped with those derived from F_2 plants of the genotype *aaBbcc* and *aaBBCc*. There are 11 F_3 families which have plants in only the fourth period of class II and in which the percentage of plants ripening in period 4 varies from 4.3 to 38.9. By removing the 9 having less than 10 plants in the fourth period the expected proportion in classes II and III becomes 485:291 and the obtained is 467:309. The *P* value calculated from the χ^2 test for goodness of fit lies between 0.20 and 0.10.

It remains to be seen whether these nine F_3 families could be placed among those determined to be of the genotypes *aaBBCC* and *aabbcc* without disturbing too greatly the goodness of fit between the theoret-

ically expected and the obtained. The data primarily affected would be those in table 5 and those shown opposite *AaBbcc* and *AaBBCc* in table 8. The nine families taken from among those of the *aaBbcc* and *aaBBCc* genotypes would fall in table 5. This would increase the obtained number of F_3 families of genotypes *aaBBCC* and *aabbcc* to 18. The expected number was found to be 11.88. By application of the χ^2 test for goodness of fit, the *P* value is still larger than 0.05, giving odds of less than 19:1 that the deviations noted are significant. By placing the nine F_3 families among those of the genotype *aaBBCC* and *aabbcc*, the percentage of the plants ripening in period 4 is 7.5. Adjusting the data in table 8 opposite *AaBbcc* and *AaBBCc*, does not change the interpretation; nor does adjustment change the interpretation placed upon the data which follow. The reason for this is that the genotypes *aaBBCC* and *aabbcc* comprise a comparatively small proportion of the population and a correction for only 7.5 percent of the plants of these genotypes does not alter the conclusions reached. Therefore, since such corrections would serve no real purpose other than to add to the complications of the analysis of the data, they are not made.

By applying the formula for the standard deviation of the binomial distribution to some of these nine F_3 families, and thus obtaining a measure of variability, it can be shown that the odds against their deviating sufficiently to produce no segregates in the fourth period due to the errors of random sampling exceed 19:1. The fact that these families have some segregates in period 4 is undoubtedly due to factors for maturity other than *Aa*, *Bb*, and *Cc*. The effect of these same factors on plants having *AA* and *Aa* in their genotype has already been noted.

F_3 Families with Plants in Classes II, III, and IV

Since the *aaBBcc* genotype caused plants to ripen in class II, the *aaBBCC* and *aabbcc* genotypes in class III, and the *aabbCC* in class IV, it is obvious that F_3 families from F_2 plants of the genotype *aaBbCc* should cause plants to ripen in classes II, III, and IV. The F_3 families ripening plants in these periods only are listed in table 9. The number of families of the genotype *aaBbCc* expected is 23.75 and the number obtained was 30. The χ^2 test for goodness of fit gives a *P* value between 0.20 and 0.10. The expected proportion of plants in classes I, II, and III is 292.50 : 658.12 : 219.38 and the obtained was 383 : 584 : 203. It is obvious that the deviations of classes II and III are greater than can be accounted for by the errors of random sampling. An examination of table 9 shows that families 1434, 1367, and 1360 have a great preponderance of plants in class II. If family 1434 had 25 percent of the plants in class I its segregation would be that expected from progeny of F_2 plants of the genotype *AabbCC*, as can be seen from an examination of table 8. If families 1367 and 1360 had 25 percent of their segregates in class I, their segregation would approximate the expected for those families of the genotype *AaBbCC*. The progeny of both the *AabbCC* and *AaBbCC* genotypes according to the hypothesis set up by the previous data would have 25 percent of the plants possessing *AA* ripening in the first period. The explanation of why families 1434, 1367, and 1360 of table 9 failed to ripen plants in the first period is found in table 2. Families 1637 and 1413 of table 2 ripened 56.4 and 20.4 percent of

their plants in the second period and they are known to be of the *AA* genotype. Thus the presence of such families as 1434, 1367, and 1360 might be expected among those from F_2 plants of the genotype *aaBbCc*. Therefore, the fact that they do occur substantiates the conclusion from the previous data postulating the presence of modifying factors. It can be seen that the removal of these 3 families would give a good fit between the theoretical and obtained, and consequently the remaining families in table 9 may be considered as derived from F_2 plants of the genotype *aaBbCc*.

TABLE 9.—*F_3 families having segregates within the second to the fourth period, inclusive; within the fifth to the ninth period, inclusive; and in the tenth period, F_2 genotype aaBbCc*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Segregates in tenth period
	I	II			III					IV		
	1	2	3	4	5	6	7	8	9[1]	10[1]		
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Percent
1566				2	7	9	1		4	31	54	57.4
1673				1	3	7	4	6	4	10	35	28.6
1495			1	1	7	5	4		5	9	32	28.1
1646		1	2	7	7	4	2		5	7	35	20.0
1511				2	7	3	3		6	5	26	19.2
1603				3	7	14	11	6	7	11	59	18.6
1648				3	6	4	2	4	8	6	33	18.2
1434		12	17							6	35	17.1
1470		1	1	17	7	4	2		6	7	45	15.6
1345			4	11	6	1	2	3	2	5	34	14.7
1357				7	2	2		3	4	3	21	14.3
1677			1	4	5	8	3	4	6	5	36	13.9
1337				9	6	5	2	1	3	4	30	13.3
1693		2	2	7	13	6	2	5	16	8	61	13.1
1636			2	7	19	8		2	3	6	47	12.8
1391			4	4	5	2	4	2	7	4	32	12.5
1659				14	4	2	6		3	4	33	12.1
1619		2	8	12	11	3			1	5	42	11.9
1415			3		7	6	5		4	3	28	10.7
1325		2	1	14	12	3	2		4	4	42	9.5
1436			4	26	10		1			4	45	8.9
1367		13	12	6		1	2		1	3	38	7.9
1402				9	6	5	3		5	2	30	6.7
1417			1	15	6	12	6		3	3	46	6.5
1390			2	6	10	6	3	1	5	2	35	5.7
1684				5	16	4	7	2	4	2	40	5.0
1505			2	6	10	8	3	4	6	2	41	4.9
1424		1	5	16	8	11	2	2	3	2	50	4.0
1349		2		17	9		4		2	1	35	2.9
1360		18	15	11		1		1	3	1	50	2.0
Total		54	87	242	216	144	86	46	130	165	1,170	

[1] Period 9 includes plants headed but not ripe on Sept. 14, and period 10 includes plants not headed on that date.

The data in table 9 furnish rather conclusive proof of the postulations drawn from table 3 that as regards habit of growth the interaction between *C* and *c* in the *aabbCc* genotype is such that the effect of *aabbCc* more nearly approaches that of *aabbCC* than it does that of *aabbcc*.

F_3 Families with Plants in Classes I, II, and IV

The F_3 progenies from F_2 plants of the genotype *AabbCC* would be expected to ripen plants in classes I and II, have a few plants in period 9 of class III, and have more than 11.7 percent of the segregates in

class IV. The total distribution for the F_3 families answering this description is given opposite *AabbCC* in table 8. The plants in period 9 are believed to be winter segregates, as the Hybrid 128 winter parent ripened approximately 2 percent of its plants in the ninth period. These plants, then, are included in class IV. Adjusting for the 2.6 percent of the plants of the *AA* genotype that ripen in class II, the proportion of plants in classes I, II, and IV is 67 : 157 : 64. The expected if *AabbCC* ripens its plants in the second period is 72 : 144 : 72. The *P* value calculated from the χ^2 test for goodness of fit lies between 0.50 and 0.30. The number of F_3 families expected of the genetic constitution *AabbCC* is 11.88. The number found was 8. The χ^2 test gives a *P* value falling between 0.30 and 0.20. The evidence for the assumption that these F_3 families are from F_2 plants of the genotype *AabbCC* is very cogent. The data following *AabbCC* in table 8 confirm previously drawn conclusions that *AAbbCC* causes plants to ripen in class I and *AabbCC* causes plants to ripen in class II.

F_3 Families with Plants in Classes I, II, III, and IV

The progeny from F_2 plants of the genotypes *AaBbCc*, *AaBbCC*, and *AabbCc* would be expected to have segregates in all four classes. The total distribution for these F_3 families is given opposite *AaBbCc*, *AaBbCC*, and *AabbCc* in table 8. The expected number of F_3 families from parents having either one or the other of these genotypes is 95, and the number of families found was 87, to which 6 must be added because some F_3 families, as stated in the discussion of methods, would have been incorrectly classified. The agreement between the obtained and the expected is very good.

Of the 27 different genotypes that will appear among the progeny of F_2 plants of the genotypes *AaBbCc*, *AaBbCC*, and *AabbCc* the effect of the *AaBbCc* genotype is the only one nonpredictable, and it is not entirely so. From the previous data it is known that *AaBbCc* causes plants to ripen in either the first or the second period or overlaps the two periods. It should be possible to determine the effect of the *AaBbCc* genotype from the total distribution of plants listed in table 8 opposite *AaBbCc*, *AaBbCC*, *AabbCc*.

The total distribution of plants for the classes I, II, III, and IV is 1,425:1,468:568:280. It has been shown previously that one F_3 family from F_2 plants of the genotype *AaBbCC* and five F_3 families from F_2 plants of the genotype *AaBbCc* would be expected to have no winter segregates due solely to the errors of random sampling. By correcting for these six families and making the other adjustments that have been shown necessary in the discussion of methods, the obtained numbers for classes I, II, III, and IV are 1,578:1,517:569:329, and the theoretical expected if plants of the genotype *AaBbCc* ripen in classes I and II in the ratio of 1:1 is 1,590.96:1,528.57:530.32:343.15. The χ^2 test gives a *P* value between 0.50 and 0.30. Since the behavior of the *AaBbCc* genotype was the only unknown, the data fit the hypothesis that the *AaBbCc* genotype causes plants to ripen in classes I and II in the proportion of 1:1. This proportion can be changed slightly and the data will still fit the hypothesis, but it cannot be changed to any great extent.

The data substantiate the previous assumption that *aabbCC* is the genotype of the winter parent and that 26 percent of the plants hav-

ing the genotype *aabbCc* ripen in class III. The data furnish proof for the hypothesis that *aaBbcc* and *aaBBCc* more nearly approach the reaction of *aaBBcc* than they do that of *aabbcc* or *aaBBCC*.

So far the working hypothesis assumed in a discussion of the data in table 8, following *AaBbcc* and *AaBBCc*, has not been proved. This hypothesis postulated that the genotype *AaBBCC* causes plants to ripen in the second period and that plants of the genotype *Aabbcc* ripen in the first period. The proof or disproof of this assumption is contained in the data of table 10.

TABLE 10.—*Segregation of F_3 families having plants in all classes and having more than 13.8 percent of the plants in the tenth period and less than 18.4 percent within the fifth to the ninth period, inclusive*

Family no.	Plants in weekly ripening periods dating from July 21, grouped in class—										Total plants	Plants in tenth period	Plants in the fifth, sixth, seventh, eighth, and ninth periods	Plants in first period
	I	II			III					IV				
	1	2	3	4	5	6	7	8	9[1]	10[1]				
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Percent	Percent	Percent
1610	12	7	2	------	3	------	------	------	2	6	32	18.8	15.6	37.5
1330	11	12	4	------	1	1	------	------	3	8	40	20.0	12.5	27.5
1427	16	------	15	1	------	4	------	------	1	10	47	21.3	10.6	34.0
1526	12	4	9	1	2	------	------	------	1	8	37	21.6	8.1	32.4
1382	15	5	3	1	2	------	------	------	2	9	37	24.3	10.8	40.5
1481	4	7	2	------	------	------	1	------	1	6	21	28.6	9.5	19.0
Total	70	35	35	3	8	5	1	------	10	47	214	------	------	------

[1] Period 9 includes those plants headed but not ripe on Sept. 14, and period 10 includes those plants not headed on that date.

The percentage proportions of plants from the progeny of plants of the genotypes *AaBbCc*, *AabbCc*, and *AaBbCC* are shown in table 11.

TABLE 11.—*Proportion of plants from progeny of plants of genotypes AaBbCc, AabbCc, and AaBbCC*

Genotype	Plants in class—			
	I	II	III	IV
	Number	Number	Number	Number
AaBbCc	48.4375	32.8125	14.0625	4.6875
AabbCc	37.5	37.5	6.25	18.75
AaBbCC	25	50	18.75	6.25

Whether the *Aabbcc* genotype causes plants to ripen in the first period can be determined if some F_3 families from F_2 plants of the genotype *AabbCc* can be isolated. If *Aabbcc* does ripen plants in the first period 37.5 percent of the progeny of the *AabbCc* genotype should ripen in the first period and 37.5 percent in the second period. On the other hand, if *Aabbcc* ripens plants in the second period 25 percent of the progeny from the *AabbCc* genotype should ripen plants in the first period and 50 percent in the second period. Since 3.875

(4.6875–0.8125) percent of the progeny of the *AaBbCc* F_2 plants and 6.25 percent of the progeny of the *AaBbCC* F_2 plants fall in class IV, it is possible to determine an upper limit of fluctuation for these genotypes that would be exceeded by F_3 families derived from F_2 plants of the genotype *AabbCc*. By application of the formula for the standard deviation of the binomial distribution, and using in this formula the average n of 42, it is found that an upper limit of 13.8 percent of the offspring in class IV would give odds of 39 : 1 against F_3 families from the genotype *AaBbCC* exceeding this limit, and considerably greater odds against F_3 families from the *AaBbCc* genotype doing so. To further insure that the plants listed in table 10 were from F_2 plants of the genotype *AabbCc*, all F_3 families having more than 18.4 percent of plants in class III were excluded from the table. Only 1 time in 40 would F_3 families having as high as 18.4 percent of the segregates in class III be expected to be from F_2 plants of the genotype *AabbCc*.

The obtained proportion of segregates in classes I, II, III, and IV, from F_2 plants of the genotype *AabbCc* and adjusted for the 2.6 percent of the plants of the genotypes *AA*— and *Aabbcc* that ripen in class II and the 26 percent of the plants of the genotype *aabbCc* that fall in class III, is 72 : 71 : 17 : 54. The theoretical expected is 80.25 : 80.25 : 13.37 : 40.13. The χ^2 test gives a P value between 0.10 and 0.05. According to the hypothesis that *Aabbcc* ripens plants in the second period, the expected is 53.50 : 107 : 13.37 : 40.13. The P value in this case is considerably below 0.01. In the first case the comparatively low P value is due to the poor fit in class IV. Because of the method of selecting the F_3 families this is to be expected, as the lower fluctuations were excluded by the 13.4 percent limit, thus making too many segregates in class IV. In the second case even though the fit were perfect in class IV, the P value would be greatly below the 1-percent point, and the odds against the deviations being due to the errors of random sampling are tremendous.

These results rather conclusively prove that *Aabbcc* ripens plants the first period and therefore that the working hypothesis used in the discussion of table 8 was correct.

Summary of F_3 Generation Data

The data bearing on the hypothesis that the parents of the cross of Hybrid 128 × Velvet Node are differentiated by three factor pairs and the data bearing on the identification of the behavior of the 27 different genotypes are summarized in table 12. This table gives the segregations of F_3 families and of the plants within F_3 families.

The segregation of F_3 families is a critical test of the hypothesis advanced since it gives the distribution of the F_2 generation genotypes. The χ^2 value obtained by comparing the observed and expected ratios was 14.1492. The P value lies between 0.50 and 0.30. By glancing at the figures in the column headed $\frac{(O-C)^2}{C}$ it can be seen that in no case is the fit a poor one. In fact, if a χ^2 were calculated for each genotype or group of genotypes in no case would the P value be less than 0.05.

Of the 15 distributions of plants within F_3 families, only 2 had P values less than 0.05. In each case the difficulty was with the proportions in classes II and III. In the case of the distribution grouped

under the *aaBbCc* genotype the deviations from the theoretical obtained after combining classes II and III are not too great to be accounted for by the errors of random sampling. This shows that the proportion of winter segregates fits the expected. The reason for the undue proportion of segregates in classes II and III is undoubtedly the segregation of factors for earliness other than those three factor pairs being intensively studied in this experiment. The evidence for this has been given previously in the discussion of table 8.

TABLE 12.— *χ^2 test for goodness of fit between the obtained and theoretical distribution of F_3 families and of plants segregating within F_3 families*

Genotype	Segregation	Families	$\frac{(O-C)^2}{C}$	Total plants in class—				*P* lies between
				I	II	III	IV	
		Number		*Number*	*Number*	*Number*	*Number*	
aabbCC	Obtained	6				247		
	Theoretical	5.94	0.0006			247		
AA	Obtained	82		3,410				
	Theoretical	95	1.7789	3,410				
aabbCc	Obtained	14				131	461	
	Theoretical	11.88	.4148			148	444	0.20 and 0.10.
aaBbCC	Obtained	10				316	86	
	Theoretical	11.88	.2975			301.5	100.5	0.10 and 0.05.
aaBBCC, aabbcc	Obtained	9				332		
	Theoretical	11.88	.6982			332		
aaBBcc	Obtained	6			203			
	Theoretical	5.94	.0006		203			
AaBBcc	Obtained	14		371	138			
	Theoretical	11.88	.4148	381.75	127.25			0.30 and 0.20.
AaBBCC	Obtained	14		123	297	134		
	Theoretical	11.88	.4148	138.5	277	138.5		0.20 and 0.10.
Aabbcc	Obtained	6		170		48		
	Theoretical	11.88	2.9103	163.5		54.5		0.50 and 0.30.
AaBbcc	Obtained	31		880	213	121		
	Theoretical	23.75	2.2132	917.25	191.09	114.66		0.20 and 0.10.
AaBBCc	Obtained	27		555	361	79		
	Theoretical	23.75	.4447	559.69	342.03	93.28		0.20 and 0.10.
aaBbcc, aaBBCc	Obtained	30			515	573		
	Theoretical	23.75	1.6447		680	408		.01 ?
aaBbCc	Obtained	30			383	584	203	
	Theoretical	23.75	1.6447		292.50	658.12	219.38	.01 ?
AabbCC	Obtained	8		67	157		64	
	Theoretical	11.88	1.2672	72	144		72	0.50 and 0.30.
AaBbCc, AaBbCC	Obtained	93		1,578	1,517	509	329	
AabbCc	Theoretical	95	.0042	1,590.96	1,528.57	530.32	343.15	0.50 and 0.30.

$\chi^2 = 14.1498$. *P* lies between 0.50 and 0.30.

The data in table 12 are taken as proof that the genotypes assigned to the F_2 plants giving rise to the F_3 families are correct and therefore that the segregations within the F_3 families as listed in the tables are a true measurement of the effect of the 27 different genotypes involved in this study. The effect of each genotype upon growth habit and earliness is summarized in table 14. By using table 14 and the previous 13 tables, all the conclusions drawn in a discussion of any of the tables and all the figures in table 12 can be derived.

EFFECT OF ENVIRONMENTAL CONDITIONS FOLLOWING DIFFERENT DATES OF PLANTING ON REACTION OF FACTORS DIFFERENTIATING HABIT OF GROWTH

To provide different environmental conditions, the F_2 generation was planted on three dates, February 28, April 4, and May 4. The different dates of planting made it possible to study the effect of seasonal change upon the factors differentiating winter and spring habit of growth, and at the same time it afforded an opportunity to

study the interaction of these factors and the relationship of the factors carried by the parents involved.

Plants from seedings made February 28 ripened in the first period regardless of whether they possessed factors for spring or winter habit of growth, showing that at this date of planting there was no differential response as regards the factors for spring habit of growth and those for winter.

The F_2 generation data are primarily of interest in the light of the interpretation of the F_3 generation data. The data obtained from the F_2 generation are compiled in table 13. This table shows that in the cross Hybrid 128×Velvet Node the F_2 generation supports the hypothesis that the parents are differentiated by three factor pairs. On this basis the obtained percentage of segregates in the tenth period is 3 and the adjusted theoretical expected is 3.875. This expected was obtained by taking into account the interaction between *C* and *c* in the genotype *aabbCc* such that this genotype ripened 26 percent of its plants in class III and the remaining 74 percent in class IV. The fit between the theoretical expected and the obtained is good. The Hybrid 128 parent ripened all but 2 percent of its plants in the tenth period and Velvet Node ripened 100 percent of its plants in the first period.

TABLE 13.—*Segregation of the F_2 generation planted at different dates*

Date of planting and cross	Proportion of total plants in weekly ripening periods dating from July 21										Total plants
	1	2	3	4	5	6	7	8	9[1]	10[1]	
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Num-ber*
April 4: Hybrid 128×Velvet Node	5	59	13	4	6	0	4	2	4	3	112
May 4: Hybrid 128×Velvet Node	0	3	23	22	20	6	0	1	3	22	121

[1] Period 9 includes those plants headed but not ripe on Sept. 14, and period 10 includes those plants not headed on that date.

The segregation within the F_2 generation planted May 4 is interesting. Velvet Node from this date of planting finished ripening at the end of the third period and all the plants of Hybrid 128 fell in the tenth period. Table 13 shows that 26 percent of the F_2 plants reacted like the spring parent and 22 percent like the winter parent. Statistical analyses are not necessary to show that these percentages fit the expected based on the hypothesis that the parents are differentiated by one factor pair. However, on this basis it is impossible to explain the 1 and 3 percent falling in periods 8 and 9, respectively. At this point it is desirable to consider some of the results of the F_3 generation data. The spring parent was found to be of the genotype *AABBcc* and the winter parent to be of the genotype *aabbCC*. The *AA*—factor pair was found to ripen plants earlier than the other two factor pairs combined, that is, the *aaBBcc* genotype. The *AA*—factor pair in the homozygous and heterozygous condition would account for the 74 percent of the segregates ripening earlier than the seventh period. Since the genotype *aaBBcc* was found to ripen plants considerably earlier than either the *aaBBCC* or *aabbcc* genotypes, the 4 percent of segregates ripening in periods 8 and 9 would be of the

genotype *aaBBcc*. The expected percentage on this basis is 1.5625 and the obtained is 4. It may be that some of the plants of the genotypes *aaBBCc* and *aaBbcc* fall in periods 8 and 9. In either event, due to the climatic conditions prevailing after May 4, in order for the spring factors *BB* and *cc* to express themselves both must be present and in the homozygous condition or else one at least must be homozygous and the other heterozygous. These data show that the environment existing during the growth of May 4 plantings is such as to prevent the *BB* and *cc* factor pairs in the genotype *aaBBCC* and *aabbcc* from expressing themselves. From these data it is also evident that *AA*—causes plants to ripen considerably earlier than *Aa*—.

DISCUSSION

INTERACTIONS BETWEEN FACTORS

The identification of the different genotypes permits a determination of their behavior. Their effect upon growth habit and date of ripening is shown in table 14. Before discussing the information presented in table 14, certain points pertinent to its correct analysis should be brought out. Table 14 does not attempt to show the factors for earliness other than those differentiating growth habit. It will be remembered that 2.6 percent of the plants from those genotypes listed as causing plants to ripen in class I ripened in class II, due in part to factors for earliness other than those listed in table 14. The proportions given for those genotypes having plants ripening in two classes can be varied somewhat, as is determinable from the data given in previous tables, but they cannot be altered sufficiently to change the interpretation placed upon the results.

TABLE 14.—*Classes in which the different genotypes cause plants to ripen*

Genotype	Class	Genotype	Class	Genotype	Class
AABBCC	I	*AaBBCC*	II	*aaBBCC*	III
AABBCc	I	*AaBBCc*	I and II (3:1)	*aaBBCc*	II and III (3:1)
AABBcc	I	*AaBBcc*	I	*aaBBcc*	II
AABbCC	I	*AaBbCC*	II	*aaBbCC*	III
AABbCc	I	*AaBbCc*	I and II (1:1)	*aaBbCc*	III
AABbcc	I	*AaBbcc*	I	*aaBbcc*	II and III (3:1)
AAbbCC	I	*AabbCC*	II	*aabbCC*	IV
AAbbCc	I	*AabbCc*	II	*aabbCc*	III and IV (1:2.8)
AAbbcc	I	*Aabbcc*	I	*aabbcc*	III

There are three factor pairs differentiating Hybrid 128 and Velvet Node as regards the winter and spring habit of growth. These same three factor pairs are of major importance in determining whether a plant shall be early or late. From table 14 it can be seen that the interaction between the *AA* factor pair and any other genetic constitution is such that the plant possessing *AA* in any combination falls in class I. Thus *AA* in combination with the two factor pairs *bb* and *CC* still causes plants to ripen in the earliest class. The interaction between *BB* and the *aa* and *CC* factor pairs is such that all but 7.5 percent of the plants ripen in class III. The same is true for the interaction of *cc* and the *aa* and *bb* factor pairs. The genotype *aabbCC* caused plants to ripen in class IV, which is the same reaction as that of the winter parent. These results are conclusive in showing that the

three factor pairs differentiating habit of growth are not duplicate factors as regards their effect upon earliness. *Aa* must be different from *Bb* and *Cc* since *AA* causes plants to ripen from 1 to 7 weeks earlier than does *BB* or *cc*. These results prove that *A* is epistatic to *b* and *C*, that *B* is epistatic to *a* and *C*, and that *c* is epistatic to *a* and *b*. In this case at least the multiple factors affecting the quantitative character earliness are not identical in their effect.

The data obtained are of interest in that they furnish some information concerning the nature of the interaction of the genes. For example, it is important to know whether the factors for winter habit of growth are the initiating force in producing physiological reactions that tend to make a plant behave as a winter type or if the later date of ripening of some genotypes is due entirely to the presence of less genetic material for the production of earliness. If the later date of ripening of some genotypes is due to a lack of genetic material for earliness, the reactions of the factors for winter habit of growth would not have any effect upon the physiological processes initiated by the genes for spring-growth habit. Then, according to such a hypothesis, all the differences noted between the genotypes must be accounted for on the basis of the factors for spring habit of growth and their interactions. The data in table 14 furnish evidence for determining whether the *CC* factor pair for winter habit of growth is instrumental in initiating physiological reactions that interact with those produced by the spring factor or factor pairs to determine whether a plant shall be early or late.

In this connection the behavior of plants having the genotype *aabbCc* is important. It can be seen from table 14 that *aabbCC* causes plants to fall in class IV, *aabbCc* in classes III and IV in the ratio of 1:2.8, and *aabbcc* in class III. Since class IV is composed of those plants having winter habit of growth, it is evident that in the heterozygote the winter reaction predominates. According to the hypothesis this could be interpreted as meaning that the *cc* factors are weak differentiators for spring habit of growth and so much so that in the heterozygote, in which the spring genetic material is reduced by one-half, their effect is more nearly that of the winter homozygote. Whether *cc* can be considered as a particularly weak factor under the implications of the hypothesis can be tested by comparing it with *BB*. Both the *aaBBCC* and the *aabbcc* genotypes caused plants to ripen in class III. It is evident that these results do not substantiate the postulation that *cc* is a weaker spring factor than *BB*, provided it is kept in mind that, according to the assumption, the factor *CC* for winter habit of growth is not supposed to have any influence. The hypothesis that *C* does not influence the reaction of *c* is still tenable if both *cc* and *BB* are weak spring factors. On this basis *aaBbCC* and *aabbCc* should give the same reaction, but such is not the case as the former genotype ripens all its plants in class III and most of the plants of the latter fall in class IV. Therefore to explain the reaction of the *aabbCc* genotype on the assumption that *cc* is a weak spring factor pair is not logical in the light of the behavior of all the genotypes in table 14.

It remains to be seen whether the assumption that there is an interaction between the allelomorphs will explain the behavior of the *aaBbCC* and *aabbCc* genotypes. On such an assumption *C* must be a strong factor for winter habit of growth since the reaction of the

aabbCc genotype more nearly approached that of the winter parent, and *b* must be a weak factor for winter habit of growth since *aaBbCC* reacted more nearly like the *aaBBCC* genotype. This would mean that the winter factor pairs are not exact duplicates of each other, which is consistent with the data showing that the spring factor pairs are not identical. It seems that the behavior of the *aaBbCC* and *aabbCc* genotypes can be explained on the basis of an interaction between the allelomorphs. If this hypothesis is correct *C* should interact with *c* to modify earliness in the genotypes *AaBBCc*, *AaBbCc*, *AabbCc*, *aaBBCc*, and *aaBbCc*. *AaBBCc* ripened plants somewhat later than *AaBBcc*, *AaBbCc* later than *AaBbcc*, *AabbCc* later than *Aabbcc*, *aaBBCc* later than *aaBBcc*, and *aaBbCc* later than *aaBbcc*. All the data are consistent with the hypothesis that there is an interaction between the spring and winter allelomorphs.

This raises the question as to whether there is an interaction between the nonallelomorphic spring- and winter-factor pairs. It has already been shown that *A* is epistatic to *b* and *c*, that *B* is epistatic to *a* and *C*, and that *c* is epistatic to *a* and *b*. Since *C* interacts with *c* to delay maturity, it seems probable that *C* might have a similar effect upon the other factor pairs. This premise is most strikingly borne out by the behavior of the genotype *AaBBCC* as compared with that of *Aabbcc*. *AaBBCC* ripened plants one class later than did *Aabbcc*. These results would be expected if *BB* were a weaker spring-factor pair than *cc* and the winter factors *CC* had no effect upon *A* or *B*. A comparison between plants having the genotype *AaBBCC* and those having the genotype *AabbCC* sheds some light on this problem. The distribution of plants having the genotype *AaBBCC* and those having the genotype *AabbCC* can be obtained from table 8. These distributions in percentages are shown in table 15.

TABLE 15.—*Percentage distribution of plants having genotypes AaBBCC and AabbCC*

Genotype	Plants in ripening period—		
	2	3	4
	Percent	*Percent*	*Percent*
AaBBCC	40.68	48.76	10.56
AabbCC	33.96	54.72	11.32

If the difference noted between *AaBBCC* and *Aabbcc* is due to *BB* being a weaker spring factor than *cc*, it is difficult to explain why *AaBBCC* should have the same effect as *AabbCC*. It can be explained by assuming that *CC* has a retarding effect upon the *Aa* and *BB* factor pairs but *Aa* will not allow plants to ripen later than the fourth weekly period. An assumption of an upper limit to explain the reason for there not being any cumulative effect of *Aa* with *BB* is not substantiated by the behavior of *Aabbcc*.

If *CC* has an effect upon the spring genes to which they are nonallelomorphic the behavior of plants having *aa* in their genotype should be explainable on this basis. Since a number of the genotypes caused plants to ripen in the same class, it is necessary to show their distribution as to periods in order to obtain a comparison between them. The distributions expressed in percentages are given in table 16.

TABLE 16.—*Percentage distribution within classes of plants having aa in their genotype*

Genotype	Distribution of plants in weekly ripening periods, dating from July 21, grouped into classes—										Total plants
	I	II			III					IV	
	1	2	3	4	5	6	7	8	9	10	
		Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Num-ber
aaBBcc		39.90	40.39	19.71							203
aaBbcc			21.00	56.00	23.00						200
aaBBCc			3.74	54.67	35.52	1.40	4.67				214
aaBbCc				18.93	38.54	33.89	1.33	7.31			301
aabbcc				7.45	20.43	31.27	37.66	3.19			145
aaBBCC				7.45	17.39	18.32	11.49	10.25	35.10		322
aaBbCC						4.46	32.14	10.72	52.68		224
aabbCc									26.00	74.00	
aabbCC									1.21	98.79	247

The percentage distribution for dates of heading for the heterozygous genotypes listed in table 16 were obtained by partitioning into their components the distributions of the progeny from F_2 plants having a known genotype. The method followed will be illustrated by the *aaBbCC* genotype. The effect of this genotype may be determined by partitioning the distribution of the progeny from F_2 plants into its components. The total distribution of progeny from F_2 plants of this genotype is given at the bottom of table 4 and is composed of segregates having three different genotypes: *aaBBCC*, *aaBbCC*, and *aabbCC*. The method of determining the reaction of the *aaBbCC* genotype is shown in table 17. The plants of the *aabbCC* genotype are the 86 in period 10 and may be subtracted to leave those of the genotypes *aaBBCC* and *aaBbCC*. Of the total number of plants, 402, one-fourth are expected to be of the genotype *aaBBCC*. This amounts to 101 plants. These would have the percentage distribution given after *aaBBCC* in table 16, and may be subtracted, leaving the number of plants of the genotype *aaBbCC* in each period. The distribution found is expressed in terms of percentage in table 16. In interpreting these data it is necessary to keep in mind that the factors for earliness other than those being studied would influence the distributions. This is particularly true since a relatively small number of F_3 families, 30 at most, were involved in the totals from which these calculations were made. For this reason they are chiefly of value for showing general trends of earliness or lateness. The distribution of the plants of the *aabbCc* genotype is compiled on the basis of the data previously discussed showing that 26 percent of the plants of this genotype ripened in period 9.

TABLE 17.—*Method of determining reaction of the aaBbcc genotype*

Genotype	Plants in weekly period—						Total plants
	5	6	7	8	9	10	
	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber
aaBBCC+aaBbCC+aabbCC	17	28	84	34	153	86	402
−aabbCC						−86	−86
aaBBCC+aaBbCC	17	28	84	34	153		316
−aaBBCC	−18	−18	−12	−10	−35		−101
aaBbCC		10	72	24	118		224

From table 16 it can be seen that in comparable genotypes those possessing *c* cause plants to ripen earlier than those having *C*. The genotype *aaBbcc* causes plants to ripen earlier than *aaBBCc* and *aabbcc* earlier than *aaBBCC*. This is the result expected if *C* has a retarding effect upon *BB* and *c*. Then *BB* must be a stronger spring factor than *cc*, as otherwise the effect of the genotype *aaBBCC* would be expected to approach more nearly that of *aabbCc*. This would explain why *aaBbCc* caused plants to ripen somewhat earlier than *aabbcc*, and *aaBbCC* earlier than *aabbCc*. The reaction of the different genotypes in table 16 can be explained by the hypothesis that the factor pairs for winter habit of growth have an influence upon the spring factors nonallelomorphic to them.

The facts brought out by this study as to the interaction of the genes make it possible to define spring and winter habit of growth in terms of the nature of the action of the factors involved. Goldschmidt's (*12*, *13*) physiological hypothesis which assumes that the genes differentiating sex in *Lymantria* are responsible for reaction processes carried on at different but definite rates, aids materially in the interpretation. First, the continuous graded series noted for the different genotypes in table 16 makes it apparent that the difference between the spring and winter parents in the cross Hybrid 128× Velvet Node as regards habit of growth is a matter of earliness and lateness. This is further shown by the fact that the *aabbcc* and *aaBBCC* genotypes caused plants to react as spring plants when seedings were made on February 28 and April 4, and as winter plants when seedings were made on May 4. Plants of the genotype *aabbCC* ripened plants the same time as plants of the genotype *AABBcc* when sown February 28, but gave a typical winter habit of growth when sown April 4 and May 4. Then, earliness or time of maturity is dependent upon quantity and kind of gene present. It is apparent from the behavior of the genotypes listed in table 16 that the number of genes present may be instrumental in determining whether a plant shall be early or late, as those genotypes with the largest number of genes for earliness matured first. The fact that the kind of gene is of primary importance is shown by *AA*, which was found to be more efficient in causing early maturity than *BB* and *cc*, and by *CC*, which has been shown to delay maturity. Then, according to Goldschmidt's physiological hypothesis, the reaction of *AA*, *BB*, and *cc* can be interpreted as rate factors in development, the strength of the reaction process depending upon the initiating factor. The behavior of *CC* cannot be explained on the basis that the reaction process of this factor pair is for earliness, but rather that it tends to inhibit heading and ripening. Consequently, whether a plant heads or ripens is dependent upon the interactions between the reaction processes, those initiated by the *A*, *B*, and *c* genes tending to bring about heading and ripening and those initiated by the *C* gene tending to delay heading and ripening even when *C* is in the presence of *A*, *B*, and *c*. The environmental conditions under which the plants are grown play a major role in determining the reaction processes of the different genes.

RELATION BETWEEN THE POLYPLOID THEORY OF THE ORIGIN OF TRITICUM VULGARE AND THE FACTORS DIFFERENTIATING HABIT OF GROWTH

The polyploid theory as to the origin of *Triticum vulgare* has a direct bearing upon the conclusions to be derived from this study. There has been considerable evidence amassed by different investigators in

the fields of taxonomy, cytology, and genetics tending to support the theory. Percival (*26*), from his extensive studies on the systemics and general characteristics of species and types of wheat reached the conclusion that *T. vulgare* is of hybrid origin, having in its parentage species of the emmer group and species of *Aegilops*. He may be quoted as follows:

> While the characters of the wheat of the small Spelt and Emmer series can be traced with reasonable certainty to their respective prototypes, from which they appear to have been derived chiefly by mutation, selection, and cultivation, investigation of the morphological features of practically all known forms of *T. vulgare* has convinced me that there is not, nor has there ever been, a prototype of the Bread Wheat series. The characters of *T. vulgare* and its allies appear to me to be those of a vast hybrid race, initiated long ago by the crossing of wheats of the Emmer series with species of *Aegilops*, and that *T. spelta* is a segregate of this hybrid.

The einkorn, emmer, and spelt groups of wheat were found by Sakamura (*29*) to have 14, 28, and 42 chromosomes, respectively. If Percival is correct in his conclusions, *Triticum vulgare* arose through hybridization of forms having a lower chromosome number and represents the higher number of a polyploid series, and these results indicate that the chromosomes of bread wheats compose 3 groups of 7 pairs. The investigations of the behavior of the chromosomes during meiosis of species crosses of *Triticum* by Sax (*31*), Kihara (*18*), Watkins (*40, pt. 1*), Thompson (*36*), and Thompson and Hollingshead (*37*) have substantiated this conclusion. These investigations together with the work of Gaines and Aase (*10*) showing that very little if any synopsis occurred during meiosis of a haploid plant derived from a 42-chromosome parent, are strong evidence in favor of the contention that *T. vulgare* is an allopolyploid. The relationship of these 3 groups of 7 chromosome pairs with the chromosomes of other species of *Triticum* and with some species of *Aegilops* has been studied further by Aase (*3*). On the basis of chromosome affinities it was found that the three groups of chromosomes were different, and that they could be accounted for by the chromosome complements found in the emmer wheats and *Aegilops cylindrica*. One of the groups of seven found in emmer was homologous with those of einkorn. These results support the theory of Percival (*26*) that *T. vulgare* is of hybrid origin and has in its parentage species of emmer and *Aegilops*. Winge's (*43*) hypothesis that polyploid series arise through hybridization and somatic doubling in the zygote, together with Rosenberg's (*28*) hypothesis and Kihara and Katayamas (*19*), gives an adequate explanation as to the mechanism by which the polyploid series could have arisen.

The evidence supports the theory that the 3 groups of 7 chromosome pairs that are found in *Triticum vulgare* existed in 3 related species each having 7 chromosome pairs. These three species were closely enough related to cross and therefore must have had some duplication of genetic material. Since, according to the polyploid theory, *T. vulgare* has a chromosome complex composed of these 3 groups of 7, it is apparent that factor duplication would be expected.

Genetic investigations with *Triticum* show that a large number of the contrasted characters in wheat are differentiated by multiple factors. The classical example is Nilsson-Ehle's (*25*) investigations in which he found that in crosses between red-seeded and white-seeded plants the characters were differentiated by three factor pairs. The work of Stadler (*35*) on the frequency of induced mutations in species of *Triticum* is extremely interesting in this connection. He

found that *Triticum* species exposed to various durations of treatment with X-rays had different mutation rates. *T. vulgare*, *T. durum*, *T. dicoccum*, and *T. monococcum*, which have 21-, 14-, 14-, and 7-chromosome pairs, respectively, were used in the study. No mutations were obtained in *T. vulgare* ($n=21$), whereas the mutation rate per r—unit was 1.9 ± 0.5 in *T. durum* ($n=14$), 2.0 ± 1.3 in *T. dicoccum* ($n=14$), and 10.4 ± 3.4 in *T. monococcum* ($n=7$). Stadler points out that these differences in mutation rates are explainable on the basis of gene reduplication being greater in the species with the higher chromosome number. This is readily understandable when one stops to consider that if 2 factor pairs are involved the expected frequency of mutation is the probability for a single factor squared, and for 3 factor pairs it is this probability cubed. Although the genetic evidence is that to be expected on the basis of the multiple factor pairs differentiating, contrasted characters being located in different groups rather than within a single group, the proof that such a situation actually existed as regards at least one pair of contrasted characters awaited the investigations of Watkins (*40, pt. 41;*) and Watkins and Cory (*42*). By cytogenetic investigations involving crosses between *T. vulgare* and *T. turgidum*, they found the genotype of the parents to be (*ww*) *W'W'* and (*WW*). During the course of the experiment all generations from F_3 to F_6 were studied in detail, as were crosses between F_1 and both parents. The results were rather conclusive in proving that *W'W'* is located in 1 of the groups of 7 chromosomes of the *vulgare* parent that are not homologous with those of *turgidum*, whereas *WW* and *ww* were found to be located in chromosomes which synapsed during meiosis.

The evidence supporting the polyploid theory of the origin of *Triticum vulgare* is rather cogent, and, furthermore, it seems that at least 3 species having 14 chromosomes in the diploid must have taken part in the evolution of this species. It appears logical to suppose that through the processes of gene mutation, chromosomal aberrations leading to gene rearrangement and hybridization, these 3 species must have evolved from 1 common ancestor which had 7 pairs of chromosomes. This hypothesis by providing a logical explanation for the origin of the factor pairs studied in this experiment furnishes a basis for a reasonable interpretation of the factor interactions prevailing.

Figure 1 illustrates the possible origin of 3 of the 6 factors involved in this study. It is not the purpose in this diagram to give the exact time and means by which the 3 factors became differentiated, but rather to set up an illustration showing the fundamental relationships. For example, the mutations responsible for differentiating *A*, *B*, and *C* are shown as mutations from the original primary species, whereas from the interpretation that follows they could just as well have taken place at any time during the evolution of *Triticum vulgare*. Also, in the diagram the cross giving rise to the primary hexaploid species is shown as being between a tetraploid and a diploid, but so far as the considerations in this article are concerned, it could just as well be shown as arising from crosses between two tetraploids, as Percival (*26*) believes was actually the case. In either event the fundamental relationships between the factors differentiating habit of growth would not be altered.

With these points in mind, deductions may be drawn from a comparison between the relationship of the factors differentiating habit of growth and their interactions. It can be seen from figure 1 that according to the hypothesis advanced, the original factor *A* is the

forbear of all six of the genes involved in this study. The diagram does not show the evolution of *a*, *b*, and *c*, but it is easy to picture how further mutations in *A*, *B*, or *C* could give rise to these factors since the great majority of mutations that have arisen in experimental material are recessives. If the mutations took place in the primary species, 6 strains would have resulted, 3 of which are shown in figure 1. Thus a series of multiple allelomorphs would have been produced consisting of 6 factors, in which *A* would be dominant to the other 5.

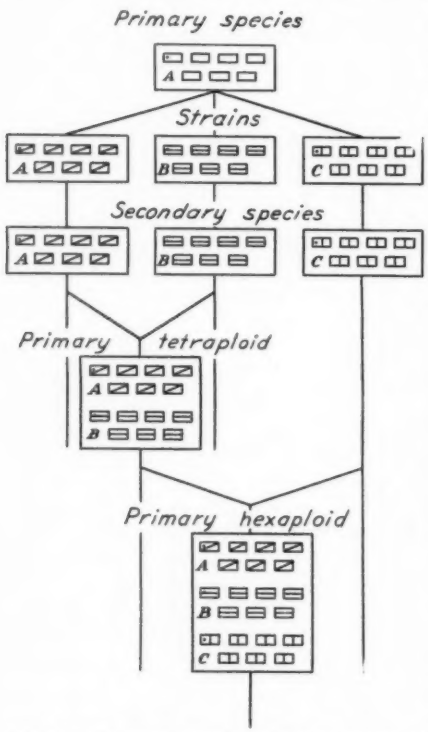

FIGURE 1.—Diagrammatic representation of the probable origin of the multiple factors differentiating habit of growth in crosses of *Triticum vulgare*. The haploid number of chromosomes is shown.

By further evolutionary changes these 3 strains could give rise to the secondary species. Then hybridization followed by somatic doubling would account for the primary tetraploid and eventually for the primary hexaploid. The *A*, *B*, and *C* factors would no longer compose an allelomorphic series, but by the duplication of chromosomes would have evolved into a series of multiple factors.

On the basis of the foregoing hypothesis an interpretation of the interactions found is interesting. *A* was found to be epistatic to the factor *C* for winter habit of growth, which is what would be expected if *A* were dominant to *C* in an allelomorphic series. For the same reason *A* would be expected to be epistatic to *b*, which was the case. Since *A* as shown by the data was only partly capable of suppressing the effects of *a*, *B* only partly capable of suppressing the effects of *b*, and *C* only partly capable of suppressing the effects of *c*, it seems that *A* might be only partly capable of suppressing the effects of the winter factors *b* and *C*. Such was proved to be the case as regards the interaction between *A* and *C*. The data show that the same relationship exists between *B* and *C*. Therefore, it would appear from these results that epistasis is not always complete, but may be only partially so, as has been found to be the case for dominance. This raises the question as to whether there is any fundamental difference between the phenomena of dominance and epistasis (*4*) as regards some series of multiple factors in polyploid species. Translocations and other cytological aberrations, provided the resulting forms became

viable, would furnish the mechanism whereby a similar relationship could have been evolved in diploids. As regards the factors differentiating habit of growth in the cross Hybrid 128×Velvet Node, the writer is unable to distinguish any fundamental difference between epistasis and dominance other than that of position.

SUMMARY AND CONCLUSIONS

The parents in the cross Hybrid 128×Velvet Node were found to be differentiated by three major factor pairs for habit of growth and earliness of maturity. Modifying factors for earliness were shown to be present. *AA*, *BB*, and *cc* were found to be factors for spring habit of growth, and their corresponding allelomorphs were found to be factors for winter habit.

The effect of each of the 27 different genotypes upon habit of growth and earliness of maturity was determined. As regards habit of growth *AA* was epistatic to *bb* and *CC*, *BB* to *aa* and *CC*, and *cc* to *aa* and *bb*.

AA was found to be a factor for early maturity, causing plants to ripen earlier than *aaBBcc*, which caused plants to be of medium early maturity. *aaBBCC* and *aabbcc* caused plants to be late in ripening, and *aabbCC* caused winter habit of growth.

The interaction between *BB* and *CC* was such that *BB* was only partially epistatic to *CC* as regards earliness of maturity, and therefore the greater earliness of *aaBBcc* as compared to *aaBBCC* is not entirely a cumulative effect.

The results furnish strong evidence that both the genes for spring habit of growth and the genes for winter habit of growth are capable of initiating physiological reactions and that the end result or character depends upon the balance between these reactions; in other words, on the balance between the genes for spring habit of growth and those for winter habit of growth under particular conditions of environment.

It was possible to obtain genotypes giving a continuous range for earliness and lateness from the early ripening of the spring parent to the winter parent in which only a few of the plants headed.

A theory for the evolution of multiple factors from a series of multiple allelomorphs and of epistasis which explains the interactions between the factors involved in this study is advanced.

LITERATURE CITED

(1) Anonymous.
1933. Evolution by selection. The implications of Winter's selection experiment. By Student. Eugenics Rev. 24: 293–296.

(2) Aamodt, O. S.
1923. The inheritance of growth habit and resistance to stem rust in a cross between two varieties of common wheat. Jour. Agr. Research 24: 457–469, illus.

(3) Aase, H. C.
1930. Cytology of Triticum, Secale and Aegilops with reference to phylogeny. Wash. State Col. Research Studies 2: 1–60, illus.

(4) Bateson, W.
1913. Mendel's principles of heredity. 402 pp., illus. Cambridge.

(5) Cooper, H. P.
1923. The inheritance of spring and winter growing habit in crosses between typical spring and winter wheats and the response of wheat plants to artificial light. Jour. Amer. Soc. Agron. 15: 15–25.

(6) East, E. M.
1910. A MENDELIAN INTERPRETATION OF VARIATION THAT IS APPARENTLY CONTINUOUS. Amer. Nat. 44: 65–82.
(7) Fisher, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.
(8) ——— Immer, F. R., and Tedin, O.
1932. THE GENETICAL INTERPRETATION OF STATISTICS OF THE THIRD DEGREE IN THE STUDY OF QUANTITATIVE INHERITANCE. Genetics 17: [107]–124.
(9) Gaines, E. F.
1917. INHERITANCE IN WHEAT, BARLEY AND OAT HYBRIDS. Wash. Agr. Expt. Sta. Bull. 135, 61 pp., illus.
(10) ——— and Aase, H. C.
1926. A HAPLOID WHEAT PLANT. Amer. Jour. Bot. 13: 373–385, illus.
(11) ——— and Singleton, H. P.
1926. GENETICS OF MARQUIS×TURKEY WHEAT IN RESPECT TO BUNT RESISTANCE, WINTER HABIT, AND AWNLESSNESS. Jour. Agr. Research 32: 165–181, illus.
(12) Goldschmidt, R.
1927. PHYSIOLOGISCHE THEORIE DER VERERBUNG. 247 pp., illus, Berlin.
(13) ———
1931. MONOGRAPHIEN AUS DEM GESAMTGEBIET DER PHYSIOLOGIE DER PFLANZEN UND DER TIERE. 528 pp., Berlin.
(14) Griffee, F.
1925. CORRELATED INHERITANCE OF BOTANICAL CHARACTERS IN BARLEY AND MANNER OF REACTION TO HELMINTHOSPORIUM SATIVUM. Jour. Agr. Research 30: 915–935, illus.
(15) Hayes, H. K.
1913. THE INHERITANCE OF CERTAIN QUANTITATIVE CHARACTERS IN TOBACCO. Ztschr. Induktive Abstamm. u. Verebungslehre 10: [115]–129.
(16) ——— and Aamodt, O. S.
1927. INHERITANCE OF WINTER HARDINESS AND GROWTH HABIT IN CROSSES OF MARQUIS WITH MINHARDI AND MINTURKI WHEATS. Jour. Agr. Research 35: 223–236.
(17) Immer, F. R.
1927. THE INHERITANCE OF REACTION TO USTILAGO ZEAE IN MAIZE. Minn. Agr. Expt. Sta. Tech. Bull. 51, 62 pp., illus.
(18) Kihara, H.
1924. CYTOLOGISCHE UND GENETISCHE STUDIEN BEI WICHTIGEN GETREIDEARTEN MIT BESONDERER RÜCKSICHT AUF DAS VERHALTEN DER CHROMOSOMEN UND DIE STERILITÄT IN DEN BASTARDEN. Mem. Col. Sci., Kyoto Imp. Univ. (B) 1: 1–200, illus.
(19) ——— and Katayama, Y.
1931. GENOMANALYSE BEI TRITICUM AND AEGILOPS III. ZUR ENTSTEHUNGSWEISE EINES NEUEN KONSTANTEN OKTOPLOIDEN AEGILOTRICUM. Cytologia 2: 234–255, illus.
(20) Lindstrom, E. W.
1926. HEREDITARY CORRELATION OF SIZE AND COLOR CHARACTERS IN TOMATOES. Iowa Agr. Expt. Sta. Research Bull. 93, pp. [99]–128, illus.
(21) ———
1928. LINKAGE OF SIZE, SHAPE, AND COLOR GENES IN LYCOPERSICUM. Ztschr. Induktive Abstamm. u. Vererbungslehre 46: 61–62.
(22) ———
1929. FRUIT SIZE AND SHAPE GENES ON THE FIRST CHROMOSOME OF THE TOMATO. Iowa Acad. Sci. Proc. 36: 189–190.
(23) ———
1931. GENETIC TESTS FOR LINKAGE BETWEEN ROW NUMBER GENES AND CERTAIN QUALITATIVE GENES IN MAIZE. Iowa Agr. Expt. Sta. Research Bull. 142, pp. 251–288.
(24) McKinney, H. H., and Sando, W. J.
1933. EARLINESS AND SEASONAL GROWTH HABIT IN WHEAT. Jour. Heredity 24: 169–179, illus.
(25) Nilsson-Ehle, H.
1909. KREUZUNGSUNTERSUCHUNGEN AN HAFER UND WEIZEN. (TEIL I). Lunds Univ. Årsskr. (n.f.) Afd. 2, Bd. 5, no. 2, 122 p.

(26) PERCIVAL, J.
1921. THE WHEAT PLANT; A MONOGRAPH. 463 pp., illus. London.
(27) QUISENBERRY, K. S.
1931. INHERITANCE OF WINTER HARDINESS, GROWTH HABIT, AND STEM-RUST REACTION IN CROSSES BETWEEN MINHARDI WINTER AND H-44 SPRING WHEATS. U.S. Dept. Agr. Tech. Bull. 218, 46 pp., illus.
(28) ROSENBERG, O.
1926–27. DIE SEMIHETEROTYPISCHE TEILUNG UND IHRE BEDEUTUNG FÜR DIE ENTSTEHUNG VERDOPPELTER CHROMOSOMENZAHLEN. Hereditas 8: [305]–338, illus. [English summary pp., 336–337].
(29) SAKAMURA, T.
1918. KURZE MITTEILUNG ÜBER DIE CHROMOSOMENZAHLEN UND DIE VERWANDTSCHAFTSVERHÄLTNISSE DER TRITICUM-ARTEN. Bot. Mag. [Tokyo] 32: [150]–153.
(30) SAX, K.
1922. STERILITY IN WHEAT HYBRIDS. II. CHROMOSOME BEHAVIOR IN PARTIALLY STERILE HYBRIDS. Genetics 7: [513]–552, illus.
(31) ———
1923. THE RELATION BETWEEN CHROMOSOME NUMBER, MORPHOLOGICAL CHARACTERS AND RUST RESISTANCE IN SEGREGATES OF PARTIALLY STERILE WHEAT HYBRIDS. Genetics 8: [301]–321, illus.
(32) ———
1923. THE ASSOCIATION OF SIZE DIFFERENCES WITH SEED-COAT PATTERN AND PIGMENTATION IN PHASEOLUS VULGARIS. Genetics 8: [552]–560.
(33) SIRKS, M. J.
1925. THE INHERITANCE OF SEEDWEIGHT IN THE GARDENBEAN (PHASEOLUS VULGARIS). I. Genetica 7: [119]–169, illus.
(34) SPILLMAN, W. J.
1909. THE HYBRID WHEATS. Wash. Agr. Expt. Sta. Bull. 89, 27 pp., illus.
(35) STADLER, L. J.
1929. CHROMOSOME NUMBER AND THE MUTATION RATE IN AVENA AND TRITICUM. Natl. Acad. Sci. Proc. 15: 876–881.
(36) THOMPSON, W. P.
1926. CHROMOSOME BEHAVIOR IN TRIPLOID WHEAT HYBRIDS. Jour. Genetics 17: 43–48, illus.
(37) ——— and HOLLINGSHEAD, L.
1927. PREPONDERANCE OF DICOCCUM-LIKE CHARACTERS AND CHROMOSOME NUMBERS IN HYBRIDS BETWEEN TRITICUM DICOCCUM AND TRITICUM VULGARE. Jour. Genetics 17: 283–307.
(38) VAVILOV, N. I., and KOUZNETSOV, E. S.
1921. ON THE GENETIC NATURE OF WINTER AND SPRING VARIETIES OF PLANTS. 25 pp., illus. Saratov. [In Russian, English résume, pp. 23–25. Reprinted from Izv. Agron. Fakult Saratovsk Univ., no. 1, 1921.]
(39) WARWICK, B. L.
1932. PROBABILITY TABLES FOR MENDELIAN RATIOS WITH SMALL NUMBERS. Tex. Agr. Expt. Sta. Bull. 463, 28 pp.
(40) WATKINS, A. E.
1924. GENETIC AND CYTOLOGICAL STUDIES IN WHEAT. I, IV. Jour. Genetics 14: [129]–171, illus., 1924; 19: [81]–96, illus., 1927; 25: 55–90, 1931.
(41) ———
1930. THE WHEAT SPECIES: A CRITIQUE. Jour. Genetics 23: 173–263, illus.
(42) ——— and CORY, F. M.
1931–32. GENETIC AND CYTOLOGICAL STUDIES IN WHEAT. Jour. Genetics 25: 55–90.
(43) WINGE, O.
1917. THE CHROMOSOMES: THEIR NUMBER AND GENERAL IMPORTANCE. Compt. Rend. Lab. Carlsberg 13: [131]–275, illus.

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WOUND INFECTION AND TISSUE INVASION BY PLASMODIOPHORA BRASSICAE[1]

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INTRODUCTION

Our earliest knowledge of the parasite concerned with the clubroot disease of Cruciferae goes back to the the studies of Woronin, which began in 1873. His first published account of the disease appeared in 1875 (*6*)[3], and the complete description of the organism (*Plasmodiophora brassicae* Wor.) and its intimate relation to the host was published in 1878 (*7*). Woronin was unable to find the myxamoebae actually pressing their way into the host cells, but implied that initial penetration took place through root hairs and epidermal cells of the primary root. He was of the opinion that migration of the plasmodia within the host tissue took place directly through the cell walls, and that a decided irritation of the invaded tissues extended well beyond those cells actually occupied by the parasite. The next important study of the parasite and its relation to its host was made by Nawaschin (*4*), who worked on diseased material sent to him by Woronin. He started his cytological study in 1893, but made little progress until 1897, when he adopted the fixing and staining methods of Flemming. He did not observe the passage of the parasite from cell to cell within the host tissues, and believed, therefore, that migration of the plasmodia by direct penetration of the cell walls did not take place. He concluded that the organism spread through the tissue by the multiplication of the invaded cells and the consequent division of the plasmodia between the daughter cells.

Lutman (*3*) made no attempt to ascertain the manner of initial infection, but believed that the parasite entered either through the young thin-walled epidermal cells or the root hairs. He reported migration by division of infected cells and figured direct penetration of the cell walls of the host tissue. Chupp (*1*) was of the opinion that direct infection occurred only through root hairs, and that direct penetration of the epidermal cells of the cabbage root did not take place. He confirmed Lutman's observation of migration of plasmodia from cell to cell by penetration of the cell walls.

Kunkel (*2*) secured infection of older root and lower stem by the application of macerated clubs to the unwounded surface. He found no evidence that primary infection took place through wounds, but was of the opinion that invasion was not necessarily confined to the

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[3] Reference is made by number (italic) to Literature Cited, p. 623.

root hairs or the primary tissues of the young rootlets. He pointed out that the plasmodia spread in the root and rootlike tissues from the point of original infection to adjacent tissues, but that they migrate rather promptly through the periderm (the primary cortex having disappeared in tissues of this age) to the cambium, where they stimulate great activity of the host cells. After reaching the cambium the plasmodia no longer penetrate tne other tissues with equal readiness, but follow the path of least resistance. In the cambium the myxamoebae increase their area of attack, since in the subsequent meristematic activity the parasite is distributed to many cells. Kunkel believed that migration in the host tissue is accomplished primarily through direct penetration of cell walls.

This paper is a report of further study of infection of the hypocotyl and stem of cabbage and related plants, and the relation of the clubroot organism to the tissues of these organs. Certain observations on root invasion are reported for comparative purposes.

MATERIALS AND METHODS

Yellows-resistant cabbage plants were employed because of the presence of *Fusarium conglutinans* Woll. in the clubroot-infested soil. Plants grown in clubroot-free greenhouse soil until approximately 90 days old were used for the inoculation experiments, which were usually conducted in a greenhouse bench. Infested soil was secured from a badly diseased field in Kenosha County, Wis. Diseased cabbage roots were also collected from this field and stored in sand until needed. Healthy cabbage roots were secure from clubroot-free soil and stored in the same manner. Certain inoculation series were conducted in naturally infested soil in the field.

Tissues were prepared for histological study by placing them in killing fluid immediately after removal from the plant. Formalin-acetic-alcohol fixative was used in the early part of the study. It was found necessary later to use a killing agent in which the tissue hardness would not be altered. A chromo-acetic-formalin solution consisting of 100 cc of 1-percent chromic acid in water, 4 cc of glacial acetic acid, and 50 cc of formalin was found to be most satisfactory when made up fresh for each fixation. When there was need of fixing relatively large pieces of tissue, incomplete fixation was avoided by subjecting the material immediately to a partial vacuum for a period of 2 to 4 hours, depending on the size of the fragments being fixed. Zirkle's (*8*) normal butyl-alcohol method of dehydration, clearing, and infiltration of tissue with paraffin was used. Embedding was done in paraffin with a melting point of about 55° C. Microtome sections were cut in thickness varying from 10μ to 15μ; a few were cut 20μ thick. A combination of safranine (1 percent in 50-percent alcohol) and fast green (1 percent in absolute alcohol) was found to be the most satisfactory stain for the structures studied. In addition, Delafield's haematoxylin was used with fair success.

EXPERIMENTAL RESULTS

INFECTION STUDIES WITH CABBAGE

During a study of soil treatment for the control of cabbage clubroot, the writer used, in one series, greenhouse-grown cabbage plants in which the internodes had become longer than usual. In setting

these plants into naturally infested soil the cotyledons and 3 or 4 of the lower leaves were removed, and the roots were placed so deeply in the soil that several of the stem internodes were below the surface. After approximately 4 weeks it was found that not only had the roots become infected but that galls had developed at the injured cotyledonary plate and on the stem at points where the leaf petioles had been removed. A careful macroscopical study of the infected tissue showed that the disease on the stems was confined to those portions which had been injured by the removal of the cotyledons or the leaf petioles. The portions that were not injured (internodes) were quite free from infection. On the stem the hyperplastic tissue took the shape of a spheroid gall, while in the roots the clubs were typically spindle-shaped (fig. 1).

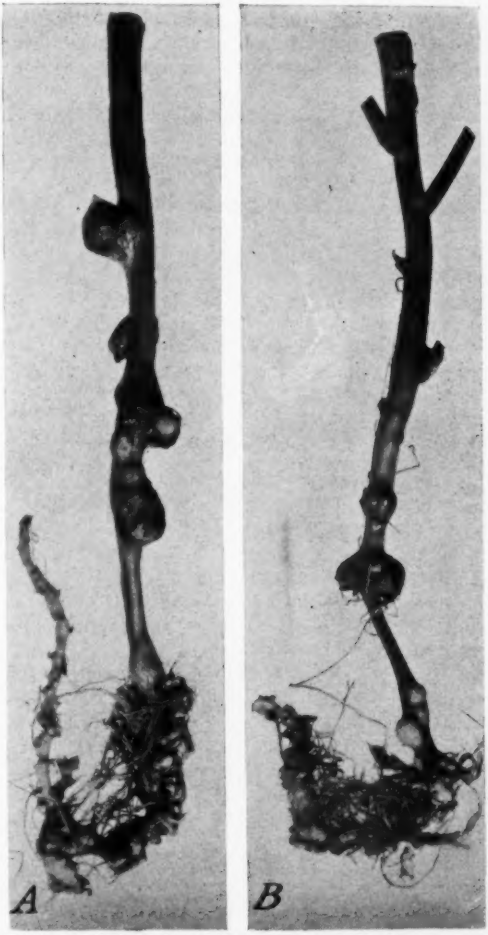

FIGURE 1.--Cabbage plants which were wounded at the lower leaf nodes and the cotyledonary node by removing the petioles at the stem, and transplanted immediately to clubroot-infested soil. The plants were 3 months old when wounded and were removed and photographed 1 month later. Note the spheroid galls which developed at the wounded nodes.

Since the foregoing observation indicated that infection of the cabbage stem occurred through wounds rather than by direct penetration of uninjured tissue as reported by Kunkel (*2*), experiments were outlined to clarify this point. Plants were divided into two groups. In 1 group the lower 4 leaves were removed by cutting the petioles at the juncture with the stem. In the second group the lower four leaves were removed by cutting the petiole 1 cm away from the stem. In the latter group the normal abscission layer at the juncture of stem and petiole formed and the remainder of the petiole dropped off in a

few days. The plants were set into clubroot-infested soil as soon as the petioles were removed. The stems were submerged so that the soil line was well above the fourth node. About 4 weeks was allowed for each experiment, since it had been determined previously that abundant clubs appeared on roots within this period.

In those plants in which the wounded stem tissue was exposed to the infested soil, infection occurred generally and the characteristic

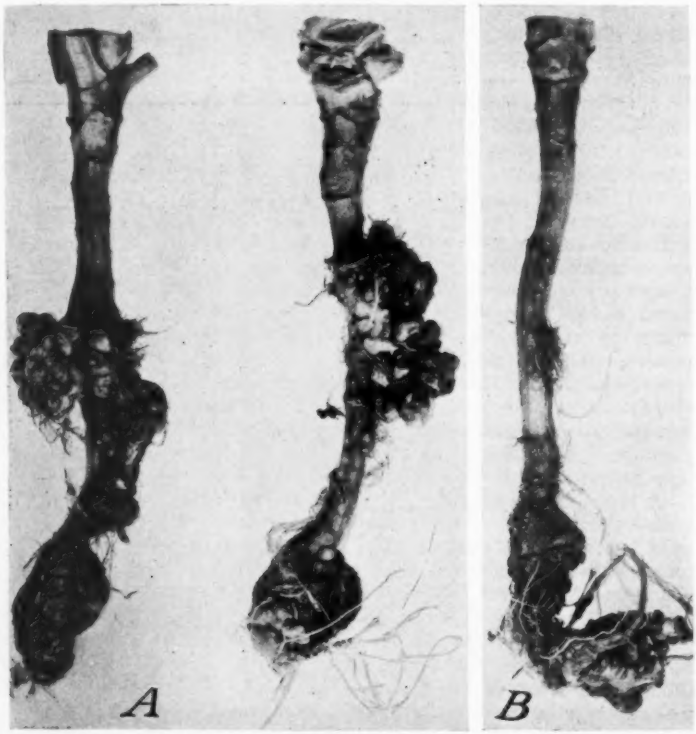

FIGURE 2.—Infection of cabbage stems in the field following injuries inflicted by the removal of leaf petioles. These plants remained in the soil for approximately 2 months. *A*, Clubs produced at the wounded nodes of the stem; secondary clubs developed on the spheroid galls as a result of infection of adventitious roots. *B*, Plant in which the stem was not injured before planting; the stem remained free from clubroot infection. Note that the root clubs developed on the injured as well as on the uninjured plants.

spheroid galls observed earlier were formed at the nodes. In those plants in which the normal abscission layer formed at the nodes only an exceptional gall developed. Histological examination of these exceptional cases showed that infection had occurred as a result of the rupture of tissue by adventive roots. In both sets of plants the usual root clubs developed, showing that there was abundance of inoculum in the soil and that the roots were infected readily. The experiment was repeated twice in the field with similar results. Figure 2 shows typical stem galls on plants wounded at the node as they were transplanted to naturally infested field soil, contrasted with a plant in the

same series in which the normal abscission layer at the node developed after transplanting.

In order to determine the relation of wound-cork formation to club-root infection, 20 plants were set aside after the removal of the leaf petioles at the nodes. After 5 days on the greenhouse bench, during which time cork formation began, they were transplanted to infested soil in the usual way. An equal number of plants were wounded at the node and planted immediately. At the end of 4 weeks, there was no evidence of infection at the nodes of plants which had been held 5 days after leaf removal, although galls were numerous on those freshly wounded at the time of transplanting. This experiment was repeated with similar results.

The possibility of infection of the internodes by way of needle punctures was next studied. Only plants free from adventive roots were used, and care was taken to avoid any other injury. Leaf petioles were severed 1 cm from the stem and needle punctures made midway between nodes in the first four internodes. The plants were set into infested soil as in previous experiments. Infection took place in the first internodes in 80 percent of the wounded areas, but in no case was it observed in the upper internodes. In the latter the punctured areas were discolored and the surrounding tissue had collapsed. The general outline of the hypertrophy of the first internode was intermediate between the spindle produced in rootlike structures and the spheroid galls produced at the stem nodes (fig. 3, *D*). Spindle formation usually did not extend more than 1 to 2 cm above or below the point of infection. In the region of injury, the gall formation was very pronounced, increasing the diameter of the cortex as much as five times the normal.

The next experiments were with the hypocotyl, which, as will be pointed out in greater detail later in the paper, is predominantly root-like in structure. Each plant was wounded with a single needle puncture about equidistant from the taproot and the cotyledonary node and set at once in infested soil. Plants which were not thus wounded served as checks. After 4 weeks the roots of all plants were generally infected. The hypocotyls became infected, however, only in those plants which had received needle wounds. The hypertrophies in this series in contrast to the spheroid galls formed at the stem nodes were typical spindles similar to those found generally on the roots (fig. 3, *A*). The unwounded hypocotyls remained free from infection (fig. 3, *B*). This experiment was repeated several times with similar results both in the greenhouse and in the field.

Kunkel (*2*) reported spindle-shaped clubs as a result of artificial inoculation of the lower stem, although the histological sections of his material indicate that he included hypocotyl in his studies. He inoculated by applying infectious debris (diseased root tissue) to a given point on the stem. When this method was used by the writer, formation of spindles occurred when the inoculum was applied to the hypocotyl or the first nodes (fig. 3, *E* and *F*), but was unsuccessful when applied to the second or higher nodes. When infested soil instead of debris was applied at a given focus no infection occurred unless needle wounds were supplied.

Kunkel's results led him to believe that infection of the stem occurred readily without the presence of wounds. As will be shown later in the paper, the application to stem or hypocotyl of decaying root tissue,

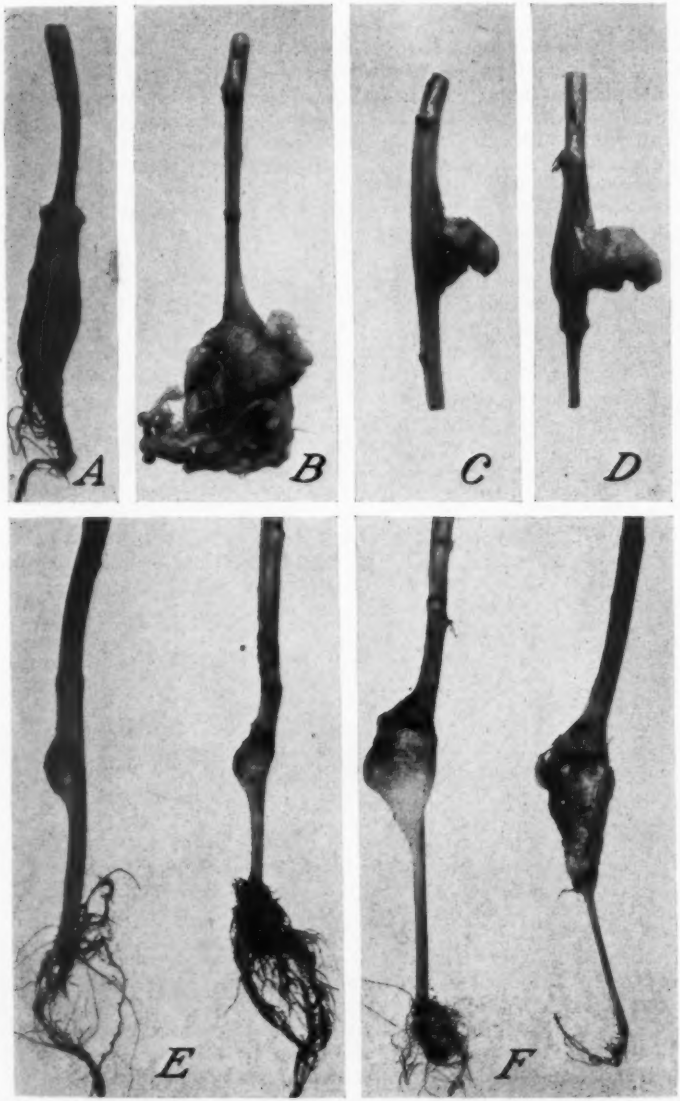

FIGURE 3.—*A*, Infection of cabbage hypocotyl following a needle wound; a spindle-shaped club characteristic of the rootlike structures developed. Note that the upper portion of the club ends rather abruptly at the cotyledonary plate. *B*, Root clubs formed on cabbage in naturally infested soil. The hypocotyl was not wounded and remained uninfected. *C*, Spheroid gall formed on an injured stem node. *D*, Infection of the first internode following a needle wound. The hypertrophy is intermediate between the spindle and gall types. *E* and *F*, Cabbage plants infected following inoculation by Kunkel's method. The spindle-shaped clubs are thickest at the point to which the infectious material was applied. *E*, Photographed 20 days after the inoculum was applied, and *F*, after 30 days.

whether clubroot-infested or not, has a corrosive effect upon the tissue and provides an unnatural opening for the parasite. The writer's experiments show conclusively that infection of the stem and hypocotyl of cabbage by the clubroot organism does not occur through the unwounded surface. However, the parasite may invade readily through the injured stem nodes and through wounds on the hypocotyl or first internode. The hypertrophy on the stem is spheroid, while on the hypocotyl it tends to be spindle-shaped as in the root proper.

INFECTION STUDIES WITH RADISH AND TURNIP

Several varieties of radish (*Raphanus sativus* L.) and turnip (*Brassica rapa* L.) were sown in naturally infested soil in the field. These species resemble cabbage in the early seedling stage, but they differ in the fact that the storage tissue forms in the hypocotyl and in some varieties in the upper portion of the taproot. Two general types of radish and turnip varieties occur. In those known as the globe and semiround varieties the edible storage tissue consists chiefly of enlarged hypocotyl. In the long or icicle varieties the storage tissue consists of hypocotyl and a certain amount of the upper taproot. In the globe and semiround varieties there are practically no secondary roots on the enlarged storage organ except at the base in proximity to the primary root; in the long varieties the secondary root zones extend from a considerable distance toward the crown of the storage organ.

A comparison of radish and turnip varieties grown in clubroot-infested soil reveals an important relation between the morphology of the storage organs and infection. All radish varieties tested were susceptible. However, in the globe and semiround varieties the clubs were confined to the unenlarged taproots (fig. 4, *B*). In the icicle varieties the lower portion of the storage organ, where secondary roots occurred, was commonly invaded and hypertrophied (fig. 4, *A*). In all radish varieties the hypocotyl tissue remained uninvaded until the latter part of the growing period. If the plants were allowed to remain in the infested soil beyond the usual edible stage, delayed infection of the hypocotyl sometimes occurred and slightly raised blisterlike lesions resulted, but large galls never formed (fig. 4, *C*). However, infection of the hypocotyl was secured readily as in cabbage by wounding the growing tissue in infested soil (fig. 4, *D*).

The same general relation was found in turnip between morphology of the storage organ and infection. In globe varieties the clubs were confined to the regions were secondary roots occur, i.e., the root proper and the base of the storage organ; in the long varieties, the lower portion of the storage organ was commonly invaded (fig. 4, *E* and *F*).

NORMAL ANATOMY OF CABBAGE

In view of the fact that these studies are concerned with the invasion by *Plasmodiophora brassicae* of the lower stem and root of cabbage, the anatomy of these tissues will be described briefly. Germination in cabbage is epigeal. As the hypocotyl expands the cotyledons appear above ground. As pointed out by Woronin (*6*) and Smith and Walker (*5*), the primary tissues of the root possess a diarch protostele with exarch arrangement of the xylem and two alternate

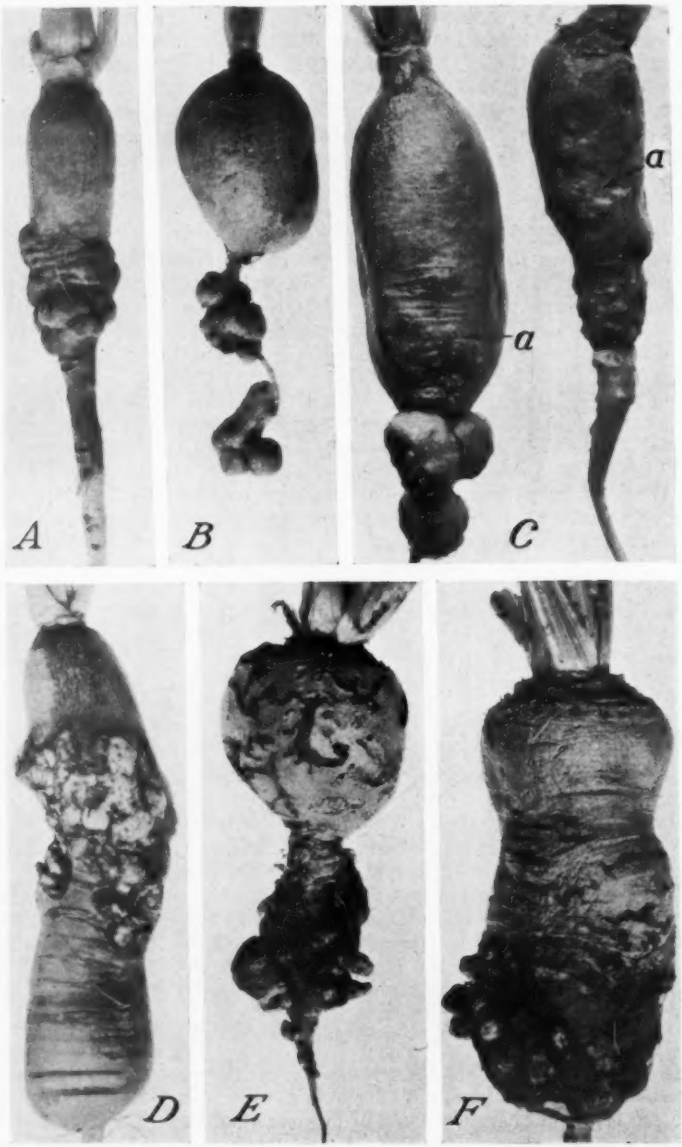

FIGURE 4.—Radish and turnip varieties grown in clubroot-infested soil: *A*, Icicle type of radish in which infection is confined to the enlarged taproot in the region of secondary laterals. *B*, Infection of taproot of globe type of radish, note that the fleshy hypocotyl is free from infection. *C*, Blisterlike areas on fleshy portion of hypocotyl (*a*) resulting from late infection. *D*, China Rose Winter radish; infection following injury to upper portion of fleshy hypocotyl. *E*, Shogoin variety of turnip, globe type; infection is confined to the taproot. *F*, Cow Horn variety of turnip, oblong type; infection is general in the fleshy taproot.

prominent strands of primary phloem. The pericycle is clearly distinguishable just outside the vascular elements. The next outer layer is the endodermis with Casparian strips sometimes discernible. The cells of the inner layer of the cortex possess characteristic thickenings which appear first in the radial walls. Concomitant with the loosening and sloughing of the cortex is the development of the permanent periderm, which arises from the tangential division of pericycle cells. This tissue replaces the primary cortex as a protective tissue, and its development takes place earlier in the root than in the hypocotyl. A cross section of the older root shows most of the tissue to be secondary, consisting of a narrow periderm surrounding the vascular stele. Most of the phloem is secondary and occupies less space than the xylem; intraxylary phloem does not occur.

In the cabbage the hypocotyl is predominantly rootlike in structure; the two exarch strands of the primary xylem of the root continue through the major portion. The first indication of hypocotyl and stem transition is the presence of a pith. The two protoxylem points are gradually separated by the parenchymatous pith cells as they approach the cotyledonary axis, and the pith cells become increasingly abundant in the upper portion of the hypocotyl. Cambium appears early in the differentiation of tissues, and the vascular elements soon appear as a continuous cylinder of secondary xylem and phloem broken by rays.

With the increase of vascular tissue, the cortex is sloughed, but persists longest in the region just below the cotyledonary axis. As in the root, tangential division of the pericycle gives rise to a prominent periderm at the periphery of which cork cambium develops. The change from the exarch condition to the endarch is rather abrupt, beginning in a majority of cases about 1 mm below the cotyledonary node and becoming practically complete at the node.

Cabbage seedlings vary as to the length of the transition region. If the hypocotyl is short the change in type of stele is rather abrupt. The strands of the cotyledonary axis supply the cotyledons, and portions of these strands appear to undergo a transition just below the cotyledonary node, each exarch trace being replaced by two endarch strands. Scattered among the phloem cells in the hypocotyl are elongated, lignified phloem fibers. These sclerenchymatous cells serve as strengthening tissue and are to be found only in the hypocotyledonary region.

The lower stem of the young cabbage plant has a dictyostele type of arrangement. The vascular bundles separated by narrow rays, enclose a conspicuous pith. The pericycle, several cells in thickness, separates the vascular tissue from the cortex. Pericycle fibers differentiate early in the maturation of the lower stem and are more prominent than in the upper stem. Cambial activity in the internodes of the lower stem soon results in a conspicuous cylinder of secondary xylem and phloem tissue. In this region the ray cells become thick-walled and a woody cylinder results. Beginning at about the second node, the medullary rays become gradually broader in the successive internodes, and a more succulent type of stem tissue is apparent. Throughout this region active division and enlargement of the parenchymatous cells of the pith take place. This proliferation, together with a limited cambial activity, is largely responsible

for the increase in diameter of the stem as the head is formed. The epidermis of the stem is somewhat cutinized and waxy.

Cabbage seedlings vary a great deal in length of hypocotyl and stem internodes. Temperature and light have a direct effect on this elongation. At the first node above the cotyledons three separate leaf traces appear and remain as distinct vascular strands in the petiole: these pass to the leaf blade without change of structure. In the region of the node the exit of leaf and branch traces leaves conspicuous gaps or interruptions in the vascular cylinder of the stem. However, they are limited in their longitudinal extent.

In normal cabbage stems, when leaf abscission occurs, a definite layer forms at the base of the petiole. Suberization of the parenchyma cells beneath this layer takes place, and a corky leaf scar is formed in which the vascular bundle scars are prominent.

Further studies of the developmental anatomy of cabbage root, hypocotyl, and stem are desirable.

PATHOLOGICAL ANATOMY OF CABBAGE

Tissue invasion by *Plasmodiophora brassicae* as well as tissue response is best determined by actual observation of the plasmodia in the structures of the host, as seen in serial sections cut both longitudinally and transversely. The relation of the parasite to the cabbage root will be discussed first.

Root Invasion

Most observations of the diseased root of cabbage plants reveal the fact that many of the clubs or overgrowths are not the irregular swellings that one might expect. If a number of diseased cabbage roots are brought together and examined it will be found that, although they may differ greatly in size, most are alike in general outline. The clubs produced on root structures are definite spindle-shaped tumors (fig. 5). When several points not very distant from one another become infected the swellings may fuse in such a way as to give rise to an irregularly shaped compound spindle. In older clubs secondary or adventitious roots arising from the club tissue may become infected and distort the original form of the spindle-shaped club.

In the diseased root of young cabbage, as seen in cross section through the thickest portion of the spindle, the position and the relative size of the primary xylem usually remain normal, since infection occurs after these tissues have differentiated and matured. Malformation is largely confined to the secondary structures, and the primary tissues are invaded only in advanced stages of infection. The most striking deviation from the normal is the presence of few and widely separated fibrovascular bundles. These xylem elements, singly or in small groups, radiate from the primary xylem. The vessels of the secondary xylem are usually smaller than in the healthy root, and are separated by abnormally broad rays, whose cells have greatly increased in size and number. The phloem can sometimes be identified, but with difficulty, by the smaller cells sometimes arranged in rows, as contrasted with the larger irregularly arranged cells of the cortex or periderm. There is no apparent continuous cambial ring as in the normal mature root. A most striking feature of the periderm and of the xylem rays is the cell groups of from four to many cells lying together which are occupied by the plasmodia of the parasite.

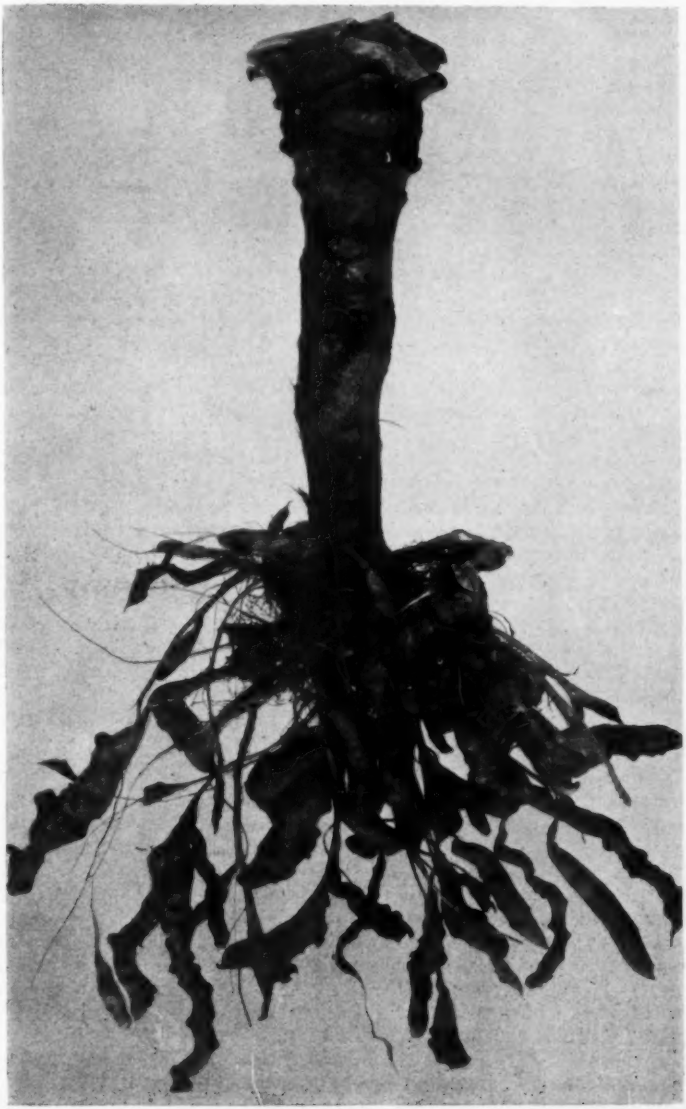

FIGURE 5.—Three-month-old cabbage, showing the characteristic spindle-shaped clubs following infection by *Plasmodiophora brassicae*. Natural field infection.

Hypocotyl Invasion

In the hypocotyl the parasite, upon entrance, migrates through the periderm and phloem rather promptly, going more or less directly to the cambium, as Kunkel (*2*) reported. There is, however, tissue stimulation and cell multiplication in the tissues outside the cambium as the parasite invades the host. It is in the cambium that progress is most rapid, and migration of the young plasmodia in this tissue is directly associated with the development of the cambial cells. Division takes place more freely in the phloem initials than in the xylem initials, and increase in the circumference of the cambial cylinder is brought about by radial and transverse division of these initials. The elements cut off on the phloem side remain undifferentiated and continue to divide and multiply.

Figure 6, *A*, represents a longitudinal section of a cabbage hypocotyl showing periderm infection through a needle puncture. The infected cells are enlarged and more numerous, while those in the uninvaded region remain normal. As a result of the increased cell activity caused by the presence of the parasite, the diseased cells are gradually pushed outwards in the radial direction by cell division beneath. Except for the tissues near the point of original invasion of the parasite, the phloem, pericycle, and periderm become infected only from the diseased cambium. As the young plasmodia infect the cambium tissue either by direct cell-wall penetration or by host-cell division, migration to the sides around the hypocotyl, as well as up and down the cambium, is directly associated with the hyperplasia in this tissue. The greatest growth stimulus is in the region of the infested cells.

Figure 6, *B*, represents a median longitudinal section of the upper hypocotyl, which is the transition region between protostele and siphonostele. The greatest hypertrophy is in the lower portion of this region of transition where protostele type of vascular arrangement occurs. The development of the spindle-shaped club is directly associated with the rapid cambial activity.

Stem Invasion

In the study of tissue invasion by *Plasmodiophora brassicae*, Kunkel (*2*) stated that the path followed by the infecting plasmodia as they passed through the tissues produced a typical spindle-shaped club in the root as well as in stem tissue. He was of the opinion that tissue hypertrophy of root and stem resulted largely from the abnormal growth of the infected cambium, and that most of the cortical tissues became infected from within. This type of tissue invasion and response is typical of the simple protostele, as is found in the root and hypocotyl of cabbage, but is not in accord with the type of response found by the writer in the first and upper nodes. Kunkel's work was, from his description and figures, largely with hypocotyl tissue, which is rootlike rather than stemlike in structure.

He reported the formation of spindle-shaped clubs on cabbage stems artificially inoculated with infectious material. The clubs produced were spindle-shaped, but they were thicker on the side of the stem to which the inoculum was sealed. As has been pointed out, the writer duplicated this type of inoculation on the hypocotyl and secured the spindle-shaped clubs (fig. 3, *E* and *F*). When infectious

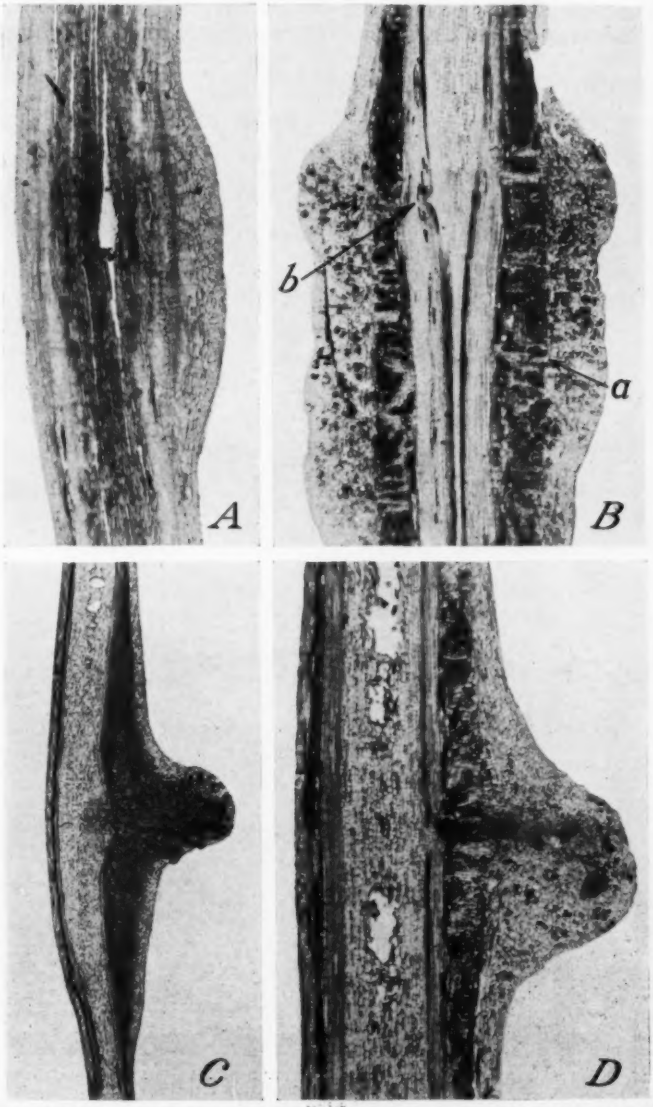

FIGURE 6.—Median longitudinal sections of cabbage plants infected with *Plasmodiophora brassicae*: *A*, Hypocotyl, showing early infection of periderm following penetration through needle wound. *B*, Diseased hypocotyl, showing region of transition between protostele and siphonostele; infection occurred through needle wound at (*a*). The hypertrophy, extending the entire length below the cotyledonary axis (*b*), has resulted largely from cell division in the stele. *C*, Infection from a needle wound in the first internode; the hypertrophy is intermediate between the spindle and the spheroid type. Advanced cortical and cambial infection is evident. *D*, Infection of second stem node, wounded by the removal of leaf petiole; the hypertrophy has resulted largely from cortical cell division.

material was applied to the first internode, infection occurred with spindle formation in the direction of the hypocotyl. When this type of inoculum was applied to the upper stem internodes no infection occurred.

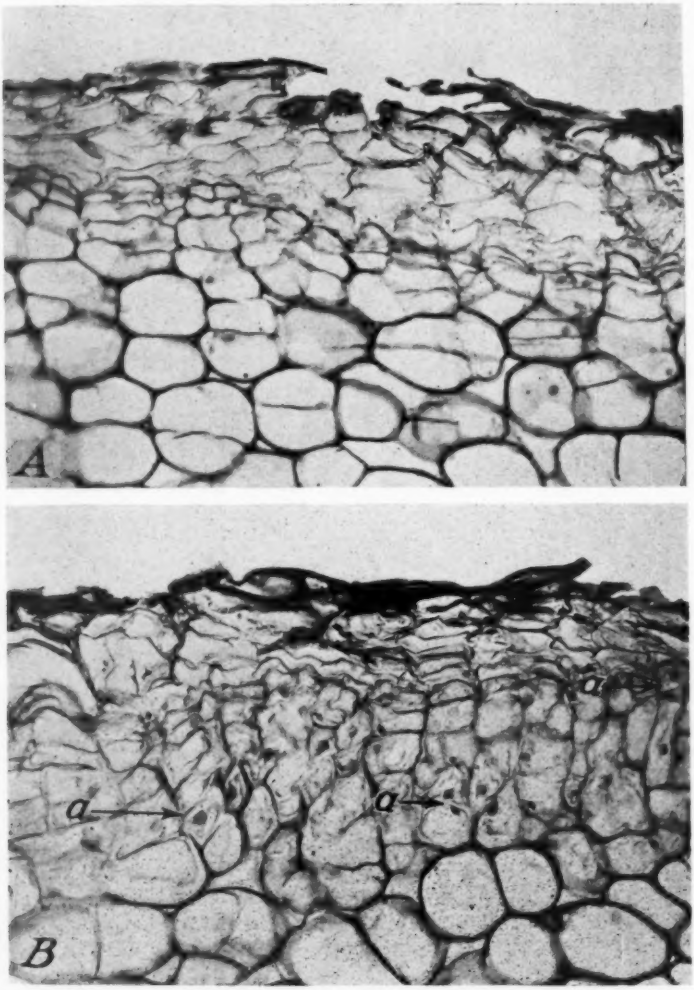

FIGURE 7.—Effect of the application of macerated cabbage root tissue to the first internode of the cabbage stem: *A*, Reaction of the tissue to the application of noninfectious material. The epidermal cells were broken down and cell division in the underlying cortex was stimulated. *B*, Reaction of the tissue to the application of macerated infectious material was similar to that in *A*. Infection has taken place and young plasmodia are below the meristematic layer (*a*). Both ×425.

Histological studies of stained sections of the first internode to which infectious as well as noninfectious material had been applied showed a very definite injury of the epidermal and underlying cells (fig. 7). The cell walls of the epidermis beneath the two types of

inoculum were very much thicker than the normal and stained readily with safranine. Many definite breaks in the epidermal walls were found and an increase in the number of cortical parenchyma cells was evident. This degenerative condition of the host cells no doubt was due to the toxic effects of the infectious and noninfectious root material.

Very early stages of host infection were observed only in tissues beneath infectious material, indicating that host infection took place through wounds resulting from the application of this type of inoculum (fig. 7, *A*). A study of stained sections of unwounded cabbage stem tissue to which diseased soil had been applied as inoculum showed no injury to the epidermal tissue, and there was no evidence of the parasite in the tissues of the host.

In the first internode with siphonostele type of arrangement of the vascular structures the cambial and cortical tissues are more equally active. This results in a hypertrophy which is intermediate between a typical spindle and a typical spheroid gall (fig. 6, *C*).

In the upper stem with a dictyostele arrangement, the plasmodia penetrate the cambium much more slowly than in the protostele (fig. 6, *D*). Migration of the plasmodia of the parasite in the cortical tissues (collenchyma and cortex) causes more proliferation than in the periderm and phloem of the hypocotyl and root. In figure 8 a cross section of a diseased node is represented where a spheroid gall was formed. The infested cortical and secondary phloem cells have divided rapidly, and tissue enlargement in this outer region has contributed very materially to the hypertrophy. The outer tissues have grown until they are many times the thickness of the same tissues in the normal stem. The hypertrophy results more from cell multiplication than from cell enlargement. Although cambial invasion takes place, hyperplasia is chiefly in the cortical tissues. The abnormal multiplication of the host cells is due chiefly to the advance of the parasite. The newly diseased cells formed in the inner tissues force the early activated tissue outward to form the spheroid gall (fig. 1 and fig. 3, *C*).

DISCUSSION AND SUMMARY

This paper presents, for the most part, an accumulation of evidence concerning wound infection and host-tissue reaction of cabbage and other crucifers to the obligate parasite *Plasmodiophora brassicae* Wor. The writer has brought together essential facts necessary to understand the normal anatomy of the plants studied, but no attempt has been made to describe exhaustively the developmental anatomy.

As previously reported by Kunkel (*2*), the spindle-shaped clubs produced on root tissues (protostele type of vascular arrangement) are the distinguishing symptom of the disease. The manner in which the parasite becomes distributed in the tissues of its host as reported by Kunkel (*2*), is characteristic of rootlike protostele structures, but is in no way the reaction observed in the upper stem regions of the cabbage.

In cabbage plants wounded either by the removal of the leaf petioles or by needle punctures, and then grown in diseased soil, club tissue was formed at the points of injury. The clubs that developed in the wounded area of the upper stem were definite spheroid galls, in contrast to the spindle-shaped clubs of the hypocotyl and

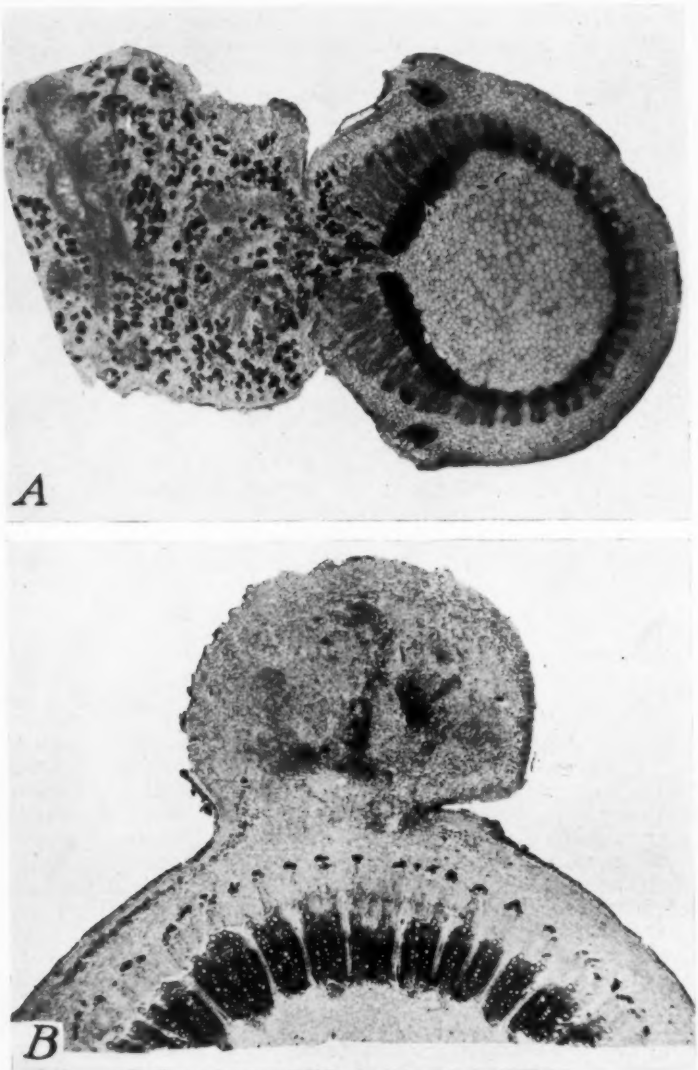

FIGURE 8.—*A*, Cross section of a spheroid gall on a cabbage stem infected through wounded leaf node; a more advanced stage than that shown in figure 6, *D*. Hypertrophy is confined almost entirely to the cortical tissues. The leaf traces can be seen in the cortex at both sides of the gall. *B*, Cross section of first internode, showing very young gall; infection through needle wound; hypertrophy confined to cortical tissues.

root. A somewhat intermediate type was produced on the first internode, a spindle with a gall at its thickest portion. Unwounded plants, as well as those wounded on the stem and allowed to callus before being exposed to the parasite, developed no club tissue on stem or hypocotyl, unless wounds were provided by the formation of adventive roots. When infested soil was applied to uninjured stem or hypocotyl no infection occurred. There was, however, host infection and diseased tissue development when macerated diseased roots were applied to the hypocotyl or the first internode of the stem. Histological studies showed a definite injury to the tissues beneath both the noninfectious and infectious material applied as inoculum and definite cell rupture with breaks or cracks in the host tissue. This evidence, together with earlier observations, indicates quite definitely that infection of hypocotyl and stem takes place through wounds or through ruptures caused by adventive roots.

The formation of a spindle-shaped club, typical of cabbage hypocotyl infection through needle wounds, is largely from abnormal growth of infected cambium, as is true of natural infection of root structures. The plasmodia migrate into the undifferentiated cells on either side of the cambium, but the greatest hypertrophy is in the cambial tissue. Cortical infection and invasion are outward from the infected cambium.

Hypertrophy of the first node (siphonostele) is intermediate between the typical spindle of rootlike structures and spheroid gall of the upper stem.

In the upper stem nodes the hypertrophy is confined largely to the cortical tissues, owing chiefly to the abnormal multiplication of the diseased host cells. The early activated tissue, forced to the outside by newly diseased cells formed as the parasite advances inwardly, results in the formation of a spheroid gall.

A comparison of radish (*Raphanus sativus*) and turnip (*Brassica rapa*) varieties grown in clubroot-infested soil revealed an important relation between the morphology of the fleshy storage organs and infection by the clubroot organism. The globe and semiround varieties of radish became infected only on the unenlarged taproots, where, as in the icicle varieties, the lower portion of the storage organ in the area of secondary roots was commonly invaded and hypertrophied. Infection of the hypocotyl of the radish was secured readily, as in cabbage, by wounding the growing tissue in clubroot-infested soil. All radish varieties tested were susceptible.

The same general relation was found in the turnip between the morphology of the fleshy storage organ and infection.

LITERATURE CITED

(1) Chupp, C.
1917. studies on clubroot of cruciferous plants. N.Y. (Cornell) Agr. Expt. Sta. Bull. 387, pp. 419–452, illus.

(2) Kunkel, L. O.
1918. tissue invasion by plasmodiophora brassicae. Jour. Agr. Research 14: 543–572, illus.

(3) Lutman, B. F.
1913. studies on club-root: i. the relation of plasmodiophora brassicae to its host and the structure and growth of its plasmodium. Vt. Agr. Expt. Sta. Bull. 175, 27 pp., illus.

(4) NAWASCHIN, S.
1899. BEOBACHTUNGEN ÜBER DEN FEINEREN BAU UND UMWANDLUNGEN VON PLASMODIOPHORA BRASSICAE WORON. IM LAUFE IHRES INTRACELLULAREN LEBENS. Flora [Jena] 86: [404]–427, illus.
(5) SMITH, R., and WALKER, J. C.
1930. A CYTOLOGICAL STUDY OF CABBAGE PLANTS IN STRAINS SUSCEPTIBLE OR RESISTANT TO YELLOWS. Jour. Agr. Research 41: 17–35, illus.
(6) WORONIN, M.
1875. DIE WORZELGESCHWULST DER KOHLPFLANZEN. Bot. Ztg. 33: 337–339.
(7) ———
1878. PLASMODIOPHORA BRASSICAE, URHEBER DER KOHLPFLANZEN-HERNIE. Jahrb. Wiss. Bot. 11: [548]–574, illus.
(8) ZIRKLE, C.
1930. THE USE OF N-BUTYL ALCOHOL IN DEHYDRATING WOODY TISSUE FOR PARAFFIN EMBEDDING. Science (n.s.) 71: 103–104.

WHITE SPOTTING IN DUROC-JERSEY SWINE [1]

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INTRODUCTION

Inasmuch as the occasional cropping out of white feet and legs in Duroc-Jersey swine has been a source of annoyance to breeders for some time, it seems desirable to attempt a study of this color impurity. When white occurs incidentally in a herd it is usually limited to the area below the pastern on any one of the four feet. The white may, however, also extend beyond the knee or hock. When it is found above the knee or hock it is usually the result of related matings. Matings were, therefore, planned with a view to analyzing the hereditary qualities that control the amount and location of white.

EARLY HISTORY OF BREED

The Duroc-Jersey breed of red swine originated in the United States during the latter half of the nineteenth century. Plumb (*17, p. 505*) [2] and Vaughan (*21, p. 529*), writing on the early history of the breed, refer to the red Spanish, Portuguese, and Guinea swine, which were imported into the United States in the first half of the nineteenth century, as important breeds in the general amalgamation that took place during the evolution of the Duroc-Jersey. Plumb further points to the old English Berkshire, which was red or sandy, as a source of red, and Evans (*5, p. 7*) ascribes the red color, in part, at least, to the influence of a pair of pigs that were imported from England in 1832. The Tamworth, also a red breed, has been mentioned by Plumb (*18, p. 395*) as a possible contributor to the red color of the Duroc-Jersey. Plumb, no doubt, refers to the Tamworth influence as effective during the general amalgamation period which took place early in the century and not to any out-crossing which may have been attempted after the Duroc-Jersey breed was established. Vaughan points to two importations of Tamworths from England that were made by Duroc-Jersey breeders in 1882 for the purpose of improving their breed. This venture, however, proved a failure and the effort was abandoned.

COLOR RELATIONS

VARIATION IN COLOR

The color of the breed varies from a light red or sandy shade to a dark red. The two breed associations [3] that have promoted the interests of the breed officially object to very dark red, very light or pale red, and black spotting. While apparently no reference is made to white spotting in Duroc-Jerseys in the "detailed qualifications"

[1] Received for publication Apr. 2, 1934; issued October, 1934. Research Paper No. 98 of the Idaho Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 633.

[3] Now consolidated into the United Duroc Record Association.

of the breed as approved by the breed associations, Evans (*4*) has made editorial comment to the effect that "we do not think a Duroc with one or more white feet or white legs should either be recorded in the records or should be allowed a place at any of the shows", and Vaughan says that "white hair anywhere in the coat is a serious objection."

ORIGIN OF WHITE AND RED

There is no reference to white in any of the breeds previously mentioned other than the English Berkshire. It is recognized, however, that these breeds were not the only influence in the foundation of the Duroc-Jersey. It is generally understood that the various imported red breeds mentioned were used on the native stock early in the nineteenth century, and that the red pigs from such matings were finally amalgamated into the strains which were brought together in the last half of that century to produce the Duroc-Jersey. The native stock, according to Spillman (*20*), was largely the result of a combined influence of breeds from all over the world. This would include such breeds as the Russian, Byfield, Big China, and Irish Grazier, that were, for the most part, all white, and others that were more or less black. It would seem that Warwick (*22, p. 251*), referring to black in the Duroc-Jersey, had this type of amalgamation in mind when he said—

> Reasoning from a genetic basis, the red of the Duroc-Jerseys may have been secured in either of two ways, depending upon the inheritance of the black. One of these is by selection of animals with smaller and smaller black spot in each generation until the animals become self red. This would assume multiple factors for black spotting. The other way is by selection of self-red animals, which probably appeared in a fairly large proportion of the progeny of the spotted ancestry.

BELTING IN THE DUROC-JERSEY

Simpson (*19*) suggests the possibility that the belting influence in swine can be referred to a very remote ancestor of the domestic hog, the Indian tapir. Spillman assumes that the belting factor in the Duroc-Jersey may have come more directly from the Chinese hog. He has also observed that white belts appear when Duroc-Jerseys are crossed with Yorkshires, a white breed, and also that belts appear when Tamworths are crossed with Chester Whites. McFhee (*11*) has found the belting tendency present in inbred Tamworths, which is a self-red breed. Spillman concludes that the belting tendency is rather prevalent in our domestic swine, particularly in those that have been influenced by the early native stock in the United States, which was more or less an amalgamation of red, black, and white breeds.

Belting in swine is limited as a rule to the fore part of the animal (figs. 1 and 2). It usually includes the shoulders and fore-rib area, however far backward it may extend. The belting that appears over the rump region of the pig in figure 3 is possibly the first of this kind on record in swine, and may have to be treated as a separate case unless it is assumed that there are location factors present in connection with belting, as appears to be the case in the location of whorls in the hair of swine (*14*) and of guinea pigs (*9*).

TWO TYPES OF WHITE

Apparently two types of white appear in the Duroc-Jersey. It is not uncommon to find the very light red or sandy shades of body color associated with almost white underlines and legs. This type of white may be the variation in intensity, referred to by Wright (*24*),

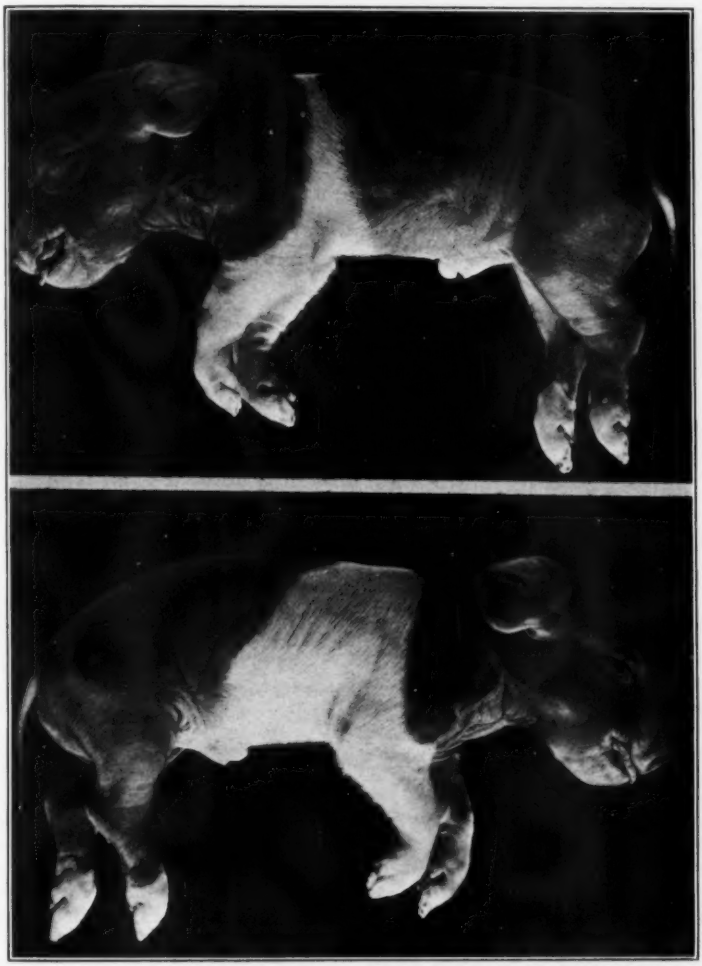

FIGURE 1.—Right and left sides of a purebred Duroc-Jersey pig (no. 16, fig. 4), the result of mating a sow that had a white left fore foot to her son that had a white left fore and left hind foot.

that determines whether the ground color should be white, sandy, or red. Wright refers also to the independent kind of variation clearly present in the white-belted swine, in which there is a white that can

appear on either a black or a red. This appears to be the same type of white as that which occurs on the feet and legs of the Duroc-Jerseys.

Lush (*21*) mentions the self-white that is dominant over all others with which it has been crossed as probably dependent upon only one factor, and the white belt (as seen in the American Hampshire or in the German Hanoverian swine) as usually dominant and depending upon at least two factors. He also refers to the white which is best illustrated by the "six white points" of the Berkshires and Poland Chinas, and which seems to be an extremely diluted red or the absence of all shades of red. The white on the extremities in the Duroc-Jerseys is apparently this latter type.

FIGURE 2.—A partial belt, complete on the left side only (*A*). Some white appears ventrally on the right side (*B*), though there is considerable space over the right side where no white appears.

VARIATIONS IN WHITE PATTERN

In all white-footed specimens described in this paper the white has invariably commenced at the extremities. However small or large the amount of white on the feet or legs, it always connected up at the foot, leaving no band of red between the white and the foot. In the two belted specimens also the white was continuous from the feet (figs. 1 and 2). In the specimen in figure 2, however, with an incomplete belt, the white was not continuous from the foot. In the revised score card of the Hampshire, a black breed with a white belt, it appears that the white may not always be continuous from the belt to the feet, as the individuals in that breed with one or both front legs black are disqualified from registration (*1*, *p. 6*).

The area covered by white was by no means fixed in these specimens. The amount of white tends to increase with the degree of concentration through inbreeding. The entire fore leg and one-third of the shoulder were often white. Likewise, white was often found over a considerable portion of the thigh. One of the extreme variations, in which white covers almost the entire right and left thigh, will be noted in figure 3. The belts that have appeared also vary in width.

PREVALENCE AND ECONOMIC IMPORTANCE OF WHITE SPOTTING

During his 30 years of experience with the Duroc-Jersey, Hanks [4] has "occasionally found a pig with 'white feet' "[5], which he says "may appear in any herd." Evans[6] states that "white feet are now stamped out of the breed except in rare cases." The strain which is represented by most of the specimens involved in this report was very popular in the show ring for a number of years, and, as a result considerable inbreeding was done, thus affording an opportunity for genetic impurities to come to light. The close breeding of other specimens of this same strain has revealed five other significant genetic impurities (*12*, *13*, *14*, *15*, *16*).

FIGURE 3.—The dam of this pig was white up to the hocks on both hind feet and legs; the sire had white on all four feet; the result of a son-to-dam mating.

For commercial production white spotting in Duroc-Jerseys has very little significance. To the breeder of purebreds, however, it constitutes a distinct genetic impurity. Breeders in general have persistently attempted to eliminate the defect, and have made progress. However, on account of the rather complex type of inheritance involved, they have not yet met with complete success.

BREEDING EXPERIMENTS

The breeding work which supplied the data for this paper was carried on, for the most part, within one rather closely bred strain (fig. 4). Some preliminary work was done for the purpose of isolating

[4] Secretary, National Duroc Record Association. Information to the author, 1934.
[5] "White feet" is often used in a general sense to include both feet and legs.
[6] Secretary, American Duroc-Jersey Record Association. Information to the author, 1934.

a few individuals that transmitted white feet. Boar D, which was self-red but which had sired pigs with white fore and hind feet, was selected for use in the test. Only one litter, however, was sired by him, in the selected matings, before he died. A son of boar D, namely E, also a self-red, was substituted for D. Three subsequent boars used were sons or grandsons of E.

Among the females used was sow no. 12, a maternal granddaughter of boar D. The sire of sow no. 12 had also sired pigs with white front feet and a few with white hind feet. In this line of matings, white predominated on the front feet. The sows subsequently used in this group of matings, which involved individuals that had the tendency to show white for the most part on the front feet, were daughters or granddaughters of sow no. 12.

In another group of matings (fig. 5) individuals were selected which showed a pronounced tendency to produce white on the hind feet, but which also produced some pigs with white on the front feet. Sow no. 1, with both hind legs white up to the hocks, was mated back to her son, boar M, which had white on both hind legs almost to the hocks and white up to the pasterns on both fore feet. Boar M had one litter mate with all four feet and much of the thighs white, and another litter mate with the left front foot white. Five other pigs in the litter were self-red. The sire and dam of sow no. 1 were self-red. The sire of boar M had a spot on top of his shoulders in which two-thirds of the hairs were white.

There were produced 196 pigs in 21 litters. Of these, 128 were self-red and 68 were marked with 1 or more white feet. The 68 pigs that were affected with white had a total of 144 white feet, 1 complete and 1 incomplete white belt over the shoulders and fore ribs, 1 belt over the rump, 3 white tail tips, and 1 small strip or blaze in the forehead. There was not an undue amount of white on the hind legs in the 3 specimens in which the tip of the tail was white. Likewise, the pig with a blaze was not excessively white on his fore legs. Of all the pigs produced in the studies, 35 percent showed white. In the son-to-dam matings, 60 percent of the pigs showed white, and in full brother-and-sister matings 50 percent showed white. It is obvious from these data that it is quite possible to increase the percentage of white.

Among the 81 pigs produced by sow no. 12, which was white on the left-front foot, there were 38, or 47 percent, with 1 or more white feet. The 38 affected pigs had 74 white feet. White appeared on the left front foot in 90 percent of the affected pigs, on the right front foot in 63 percent, and on the hind feet in only 30 percent. In no case did there appear among the pigs produced by this sow one with white on the hind feet unless there was white also on 1 or more of the forefeet.

In the group of matings involving white markings on the hind feet (fig. 5), 40 percent of the progeny of sow no. 1 had white on the hind feet and 19 percent had white on one or both fore feet. It appears from these data that the white markings tend to recur, for the most part, on the appendages which are affected in the ancestry.

The amount of white increased with the concentration of breeding. In some cases the white extended well up on the shoulders, and in a number of cases the thighs were one-half or more covered with white. The specimen in figure 3 is a good example of excessive white marking

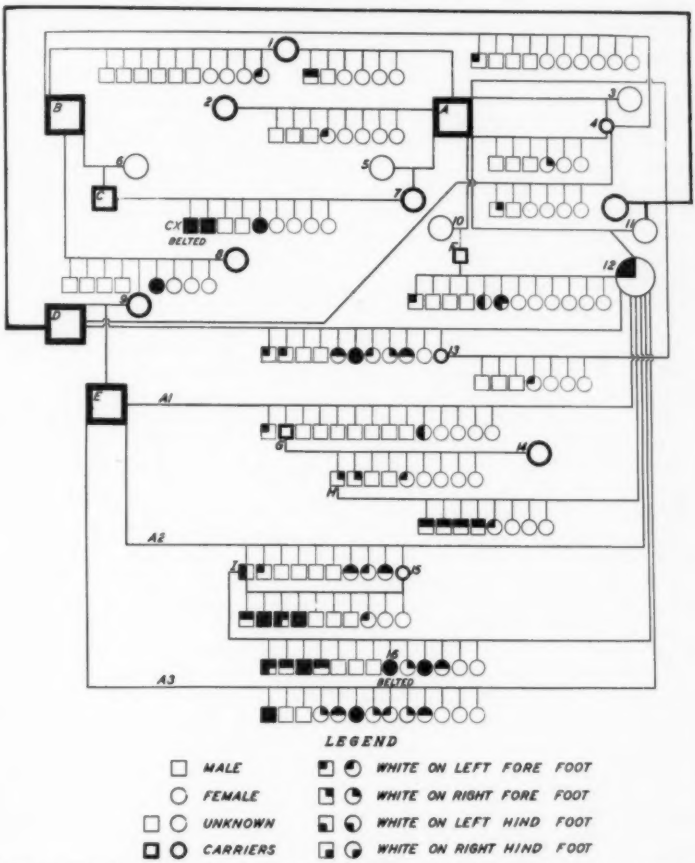

FIGURE 4.—Genealogy diagram of purebred Duroc-Jerseys in which white markings are most prominent on the fore feet.

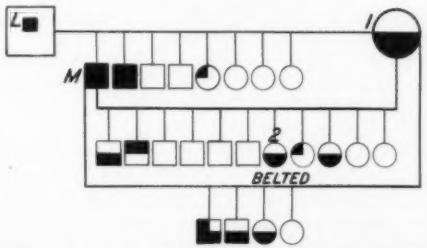

FIGURE 5.—Genealogy diagram of purebred Duroc-Jerseys in which white markings are most prominent on the hind feet.

on the thighs. There occurred almost a duplicate of this pattern in a full brother of this specimen except that the white did not come together on top of the rump.

DISCUSSION

It appears that the genetic influence for white and its occurrence on different parts of the body cannot be explained on the basis of a single set of factors. There are at least two patterns of white that must be considered, namely, the white belt and the white extremities. In both patterns a white is involved that replaces entirely the red. Both patterns vary with respect to the amount of white. This condition is also true in the Hampshire breed, in which white may appear only on the fore feet in the absence of a belt, or in the form of a very wide belt with white in large amounts on all four feet.

When white spotting is present in many of our domestic animals it tends for the most part to be limited to the appendages. This pattern is at least one that is common to horses, cattle, and swine. In the Poland China and Berkshire breeds the "six white points" are characteristic of this pattern, which Wright (*23*) describes as a reduced form of the type of white pattern that is found in the irregular splashes formerly common on the sides of these two breeds.

In addition to the red and sandy "bicolor", Wentworth and Lush (*22*) refer to three distinctly different types of white markings that are readily recognizable, one of which is the broken splotching of white "probably related genetically to the Berkshire 'six white points'." Lush suggests that the "six white points" of the Berkshire or Poland China are probably an exteremly diluted red or the absence of all shades of red. The white markings on the extremities in Duroc-Jerseys are not materially different from the "six white points" in Poland Chinas and Berkshires. It appears that the white can be increased rather indefinitely in Duroc-Jerseys. The extension of white in "hooded" rats (*3*) and in guinea pigs (*6*), and the Akhissar spotting of the house mouse (*8*) and other mammals (*2*) show clearly the effect of selective matings for increasing white. Likewise, in Poland Chinas and Berkshires the breeders are selecting to hold the white within prescribed bounds.

Spillman (*20*) and others have concluded that the inheritance of belt in the Hampshire depends on two or more factors. Lloyd-Jones and Evvard (*10*) have shown that the belt pattern is genetically complex. In all probability the belt is inherited in the same way in both breeds, whatever the final analysis of factors may reveal. In the Duroc-Jerseys as well as in the Hampshire the width of belt varies, and the pattern on both sides of the body also varies. From his studies of this variation in cattle, Ibsen (*7*) concluded that the difference in pattern on the two sides of the body is nongerminal in nature. The amount of white and the pattern of white vary also on the feet and legs. Indeed, lack of uniformity is the rule and not the exception. A number of investigators have explained that this variation in other breeds is, at least in part, due to nongenetic or developmental influences. It appears somewhat difficult, if not impossible, to explain the variations in the amount and pattern of white by a specific allelomorphic relationship between the red and white.

Wentworth and Lush (*23*) have determined that red pigment depends upon the complementary action of two factors, and from the results of their studies they conclude that both factors must be present in the Duroc-Jerseys if they are to be self-red. One of these is the factor for sandy and the other, which is a separately inherited factor, intensifies sandy to red. The absence of both of these factors results in white.

In all probability the explanation for the variations in amount, pattern, and location of white will have to be referred both to nongenetic influences and to complex modifiers as they affect the restriction for red.

The inheritance of white spotting is apparently not due to a simple recessive gene, unless there are factors that determine location which are inherited independently of the modifiers that restrict the red in such a way as to interfere with the normal ratio.

SUMMARY AND CONCLUSIONS

It has been definitely established that white occurs in purebred Duroc-Jerseys and that it may be found on the feet and legs, in the forehead, on the tail, and on the body in the form of partial or complete belts.

There is considerable variation in the amount and in the pattern of white wherever it does occur.

The inheritance that allows white to appear has, in all probability, been handed down from one or more of the early breeds or breed admixtures that entered into the general amalgamation that preceded the early development of the breed.

The amount of white increases with inbreeding and appears to be due to modifiers that influence the restriction factors which determine the amount of red. There also may be nongenetic influences which affect the pattern as well as the amount of white.

The factors for belting are independent of those for color. However, the belt cannot appear in the absence of restriction factors for red, as these apparently permit white to come into evidence.

Inasmuch as complete or partial belts have appeared on the shoulders, on the side some distance back of the shoulders, and over the rump, it would seem that there are factors present which determine the location of the belt in the Duroc-Jersey.

It appears from the data on hand that the inheritance of white spotting is not due to a simple recessive gene. Normal ratios of a somewhat more complicated inheritance seem to be masked by nongenetic variability.

LITERATURE CITED

(1) ANONYMOUS.
1933. HAMPSHIRE SCORE CARD REVISED. Amer. Hampshire Herdsman 8 (1): 6.

(2) ALLEN, G. M.
1914. PATTERN DEVELOPMENT IN MAMMALS AND BIRDS. Amer. Nat. 48: 385–412, 467–484, 550–566.

(3) CASTLE, W. E.
1924. GENETICS AND EUGENICS; A TEXTBOOK FOR STUDENTS OF BIOLOGY AND A REFERENCE BOOK FOR ANIMAL AND PLANT BREEDERS. D. 3, 434 pp., illus. Cambridge, Mass.

(4) Evans, R. B.
1930. observances of the editor. Duroc Sentinel 5 (11): 3.
(5) Evans, R. J.
1918. history of the duroc, a short history of the duroc jersey breed of swine. 83 pp., illus. Chicago.
(6) Ibsen, H. L.
1932. modifying factors in guinea pigs. Sixth Internatl. Cong. Genetics Proc. v. 2, pp. 97–101.
(7) ———
1933. cattle inheritance. i. color. Genetics 18: [441]–480.
(8) Keeler, C. E.
1933. akhissar spotting of the house mouse. Natl. Acad. Sci. Proc. 19: 477–481, illus.
(9) Landauer, W.
1929. die vererbung von haar- und hautmerkmalen, ausschliesslich färbung und zeichnung. ii. Ztschr. Induktive Abstam. u. Vererbungslehre 50: [356]–415.
(10) Lloyd-Jones, O., and Evvard, J. M.
1919. studies on color in swine. Iowa Agr. Expt. Sta. Research Bull. 53, pp. [203–208.
(11) McPhee, H. C.
1931. swine inbreeding at the united states department of agriculture—a progress report. Amer. Soc. Anim. Prod. Proc. 1930: 131–134.
(12) Nordby, J. E.
1930. congenital ear and skull defects in swine. Jour. Heredity 21: 499–501.
(13) ———
1932. inheritance of whorls in the hair of swine. Jour. Heredity 23: 397–404.
(14) ———
1933. congenital melanotic skin tumors in swine. Jour. Heredity 24: 361–364.
(15) ———
1934. kinky tail in swine. Jour. Heredity 25: 171–174.
(16) ———
1934. congenital defects in the mammae of swine with special reference to inverted nipples. Jour. Heredity (in press).
(17) Plumb, C. S.
1906. types and breeds of farm animals. 563 pp., illus. Boston, New York, [etc.].
(18) ———
1922. a study of farm animals. 551 pp., illus. St. Paul, Minn.
(19) Simpson, Q. I., and Simpson, J. P.
1908. genetics in swine hybrids. Science (n.s.) 27: 941.
(20) Spillman, W. J.
1907. inheritance of the belt in hampshire swine. Science (n.s.) 25: 541–543.
(21) Vaughan, H. W.
1931. breeds of livestock in america. 780 pp., illus. Columbus, Ohio.
(22) Warwick, B. L.
1926. inheritance of black in swine. Jour. Heredity 17: 251–255, illus.
(23) Wentworth, E. N., and Lush, J. L.
1923. inheritance in swine. Jour. Agr. Research 23: 557–581, illus.
(24) Wright, S.
1918. color inheritance in mammals. viii, swine—much evidence still necessary to clear up problems—three independent kinds of variations can be distinguished. Jour. Heredity 9: 33–38.

GENETICS OF RESISTANCE TO BACTERIAL WILT IN ALFALFA [1]

By R. A. BRINK, *professor of genetics, Wisconsin Agricultural Experiment Station;* F. R. JONES, *senior pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture;* and H. R. ALBRECHT, *assistant in genetics, Wisconsin Agricultural Experiment Station*

INTRODUCTION

In several diverse regions in the United States where bacterial wilt, caused by *Phytomonas insidiosa* (L. McC.) Bergey et al., has become a major factor in reducing the production of alfalfa, *Medicago sativa* L., there is urgent need of varieties which are resistant to the disease and, at the same time, suited in other respects to the areas involved. Various avenues which may lead to a satisfactory solution of the problems presented are being explored. Under the auspices of the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, a wide search is being conducted among American varieties and importations from Asia, Europe, and Africa for strains which will meet the new requirements, or which, at least, will provide material for the development of suitable resistant strains by more intensive methods. The possibilities offered by different systems of breeding which turn upon individual plant selections likewise are being studied. In the critical evaluation of material now in the trade and in the effective utilization of resistant individuals from various sources in developing varieties which will withstand the parasite, an understanding of the mode of inheritance of resistance would appear to be second in importance only to a sound knowledge of the disease itself.

The data on the genetic basis of resistance thus far obtained are not numerous. Nevertheless, significant evidence on certain aspects of the problem has been secured. Because of this and in view of the evident complexity of the problem and the likelihood that further progress in solving it will be slow, the results are presented now.

METHODS

The tests were all made in one season, 1933. Aside from foundation stocks, the seed used in the experiments was produced in greenhouses screened to exclude insects likely to transport pollen from plant to plant. The flowers were stripped by hand in selfing, 95-percent alcohol being used on the fingers after operating on each individual. In making crosses the ordinary precautions were observed to secure seed of known parentage. The tests were made on a fairly uniform plot of land to which water was applied as needed to maintain the plants in a thrifty condition. The seedlings were started in the greenhouse in December 1932, inoculated in the spring by scrap-

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ing the roots in a suspension of the wilt organism, and set in the field at once. The results from the check rows of Grimm and Hardistan alfalfa, treated in the same way as the test material and distributed throughout the plot, show that a uniform and relatively severe wilt infestation was secured. Among 754 Grimm check plants only 5, or 0.66 percent, were found to be uninfected on digging in the fall. The corresponding value for Hardistan, a strain known to possess considerable resistance, was 101 healthy plants in a total of 609, or 16.6 percent. The final counts are based on the number of plants living about 6 weeks after transplanting.

CLASSIFICATION OF PLANTS FOR RESISTANCE

The evaluation of individual plants for resistance at the termination of a test presents unusual difficulties. These difficulties, recently discussed by Jones,[2] appear from the evidence which he presents to arise from two circumstances: (1) Resistance seems to vary continuously among individuals, from slight to a condition approaching immunity, and (2) no single practicable test appears yet to have been devised that will distinguish precisely between individuals which may be supposed to differ only slightly in resistance. It is to be anticipated that among the environmental factors affecting the incidence of wilt in a test carried out in the field there will be a residuum of circumstances conditioning the reaction of the plant to the organism which will not have been identified and which were not under control. It may therefore be expected that more or less frequently two individuals of identical genotype and receiving similar treatment, so far as factors under control are concerned, will develop the disease in a significantly different degree. Moreover, it has been found in the practical work of classifying the plants that in some populations the character of the disease symptoms differs strikingly from that in other populations.

As a working plan, therefore, it is necessary to set up provisional classes each of which will embrace a particular segment of the presumably continuous array of reactions. Such a procedure, to be helpful, must satisfy at least two requirements: (1) The classes should be broad enough so that border-line cases, whose disposition must be more or less arbitrary, will constitute but a small part of the total; (2) each class should be predicated upon a "type" which is not only definable in terms of the results of the test applied but which also appears to have a validity beyond the conditions peculiar to the experiment. The validity sought here in classification is one which might reflect, in a general way, the probable fate of the plants under the most severe wilt conditions found in the field. It should be understood that the claim is not made that the behavior of the plants in the field would be expected necessarily to coincide with that on the wilt plot, but rather that the two would be parallel and in approximately the same order. The readings afforded by the wilt-plot test may prove to have been too high or too low as judged by the survival of plants grown in the ordinary way on wilt-infested soil; but it is believed that more drastic conditions for infection have been provided in the test than are likely to occur in the field.

[2] JONES, F. R. TESTING ALFALFA FOR RESISTANCE TO BACTERIAL WILT. Jour. Agr. Research 48: 1085-1098. 1934.

Four classes have been established, as follows: (1) Healthy, (2) slightly infected, (3) badly diseased, and (4) dead. This classification is definitive for groups 1 and 4, but does not sharply delimit the other two groups. The criterion used in distinguishing between class 2 plants, slightly infected, and class 3 plants, badly diseased, was the extent of lesions in the roots. It is assumed that under field conditions plants in the former group, if infected at all, would show a relatively high resistance, whereas those of the latter group would succumb rather readily to the disease.

EXPERIMENTAL DATA AND DISCUSSION

In the intensive search which has been conducted during recent years for a wilt-resistant alfalfa among domestic plantings, two strains, aside from the Ladak variety, have been discovered which, as compared with the common and variegated forms, are outstanding in their ability to withstand the disease. These are Hardistan[3] and Kaw.[4] Both stocks appear to be of Turkistan origin. With the primary purpose of isolating some of the most wilt-resistant components of Hardistan and, secondarily, of obtaining light on the genetic behavior of resistance, 164 plants of this variety were selfed and the offspring of the 95 individuals yielding at least 80 seeds were tested for their reaction to the disease.[5] The 95 parent plants, represented on the wilt plot by as many families, were thus a random sample of the variety, except that the lowest seed producers were not included. The average number of plants per family 6 weeks after transplanting was 45. The results of this individual plant-progeny test are shown in figure 1. It will be noted that the progenies range continuously in proportion of resistant plants (classes 1 and 2) from 0 to 100 percent. The largest single subgroup of families, comprising 19 members, contains no resistant individuals. This fraction, combined with the progenies having some, but fewer than 10 percent, resistant segregates, makes up about one-third of the total. The remaining families are distributed in a rather uniform and unbroken series. In one of the two families falling in the highest class all the plants were resistant and in the other family all were resistant but one.

The facts here presented show the highly diverse character of families from individual plants in a stock known to carry, in the aggregate, a considerable amount of resistance. The fact that plants capable on self-pollination of giving at least some resistant offspring may vary continuously over a very wide range in the proportions actually thrown shows clearly that the genetic basis of resistance is far from simple. The cross section of the Hardistan variety afforded by this series of plant-progeny tests points to a complex of germinal elements making for resistance distributed very unevenly between different individuals in the stock and interacting in ways unknown at present to produce in each generation a certain, possibly fairly constant, proportion of resistant plants. It is a reasonably safe assumption also that by rather simple methods of selection the average resistance of the population could be shifted downward to zero, on the one hand, or,

[3] KIESSELBACH, T. A., ANDERSON, A., and PELTIER, G. L. A NEW VARIETY OF ALFALFA. Jour. Amer. Soc. Agron. 22: 189–190. 1930.

[4] SALMON, S. C. KAW—A NEW ALFALFA. Jour. Amer. Soc. Agron. 24: 352–353. 1932.

[5] The stock seed used was harvested from the original Hardistan field and came to the writers through the courtesy of L. F. Graber of the Wisconsin Station, who obtained it from the Nebraska Agricultural Experiment Station.

on the other, upward well above the present means to some unknown value, which would have to be determined empirically.

Since the ultimate solution of the wilt problem demands the production of varieties which will not only withstand the disease but will also be acceptable in other regards it is important to know whether resistance is compatible with other desirable qualities. A study was made of the relation between seed production and reaction to the disease in the above-mentioned Hardistan population. Hardistan, like other strains of Turkistan origin, is less productive of seed than the commonly grown American varieties. The 163 Hardistan plants from which the 95 families in the wilt test were derived produced an average of 207 seeds each on being selfed in the greenhouse. On an adjacent bench and under almost identical conditions of light, temperature, moisture, and handling, 380 unselected Grimm plants of the same age gave a mean yield of 358 seeds. The somewhat larger size of the Grimm plants gives this variety an advantage, but the difference, amounting to about 70 percent, is almost certainly not attributable to this factor alone. Adjacent and comparable plots of Grimm and Hardistan in the field in 1933 showed a much greater disparity in seed production than was observed in the greenhouse. Whether the low seed production and relatively high wilt resistance of Hardistan and of other Turkistan alfalfas is indicative of a general relationship between these two variables is a question of considerable importance.

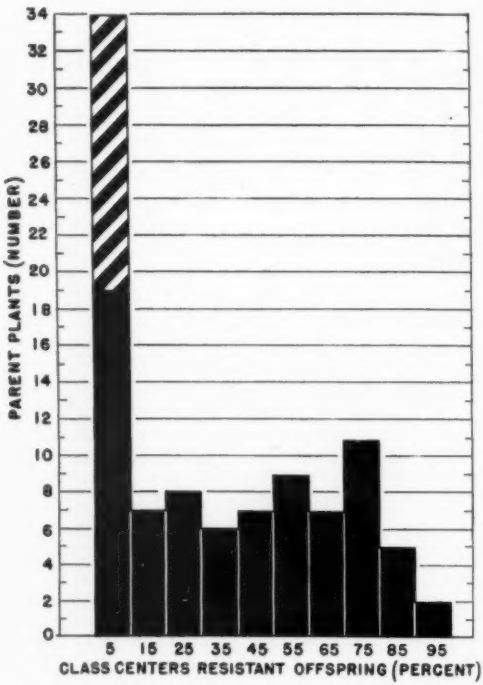

FIGURE 1.—Distribution of 95 families from once-selfed plants of the Hardistan variety, with reference to the proportion of resistant individuals in the progenies. The cross-hatched bar represents the families in which no resistant plants occurred.

The results obtained in the present study are depicted in figure 2. The 95 self-pollinated Hardistan individuals were grouped into classes according to the number of seeds produced, a uniform class interval of 200 seeds being used up to 800. The seven individuals lying above 800 and ranging up to 1,464 were lumped together at 1,100, the approx-

imate value of their mean. The average percentage of wilt-resistant offspring from the plants in each of these classes is indicated by the position of the circles with reference to the vertical scale. Reading from left to right the numbers of parent plants on which the 5 respective points are based are 42, 28, 9, 9, and 7. Since the frequency distributions for number of seeds and percentage of wilt resistance are both markedly asymmetrical there is no entirely satisfactory way of expressing in a single term the relationship between the two variables. Grouped in the way indicated, however, the data give a correlation ratio (η) of 0.29 for percentage wilt-resistant offspring on parent seed number. A finer grouping, as might be expected, leads to a somewhat higher value of η. Caution is necessary in judging the significance of this correlation ratio. There is no evident relationship between the two variables in the first four seed classes. The value of η is due, in large part, to the character of the array corresponding to the highest seed class. The seven plants producing more than 800 seeds each all proved to have very low percentages of wilt-resistant offspring. This is the most significant fact which the analysis brings to light.

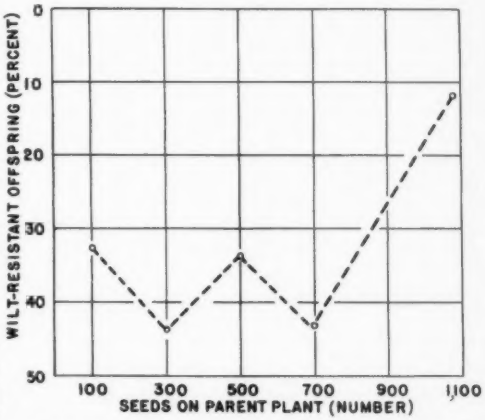

FIGURE 2.—Relationship between seed production in the parent plant and percentage wilt resistance in the offspring of 95 self-pollinated Hardistan plants.

The average percentage of wilt-resistant offspring from this group of once-selfed Hardistan plants is 14.8. This is to be compared with a value of 16.6 percent for the check rows of the Hardistan variety and 14.2 percent in a block of 1,006 Hardistan plants also included in the test. On genetic grounds it is to be expected that the average heterozygosity of the once-selfed stock would be about 50 percent less than that in the Hardistan variety. Since the large increase in homozygosity is not accompanied by a significant change in the average proportion of resistant plants it would appear that heterozygosity has little if any relation to wilt resistance. If this is true it appears probable that, other circumstances permitting, pure lines could be established by continuous inbreeding which would exhibit the various levels of wilt resistance found in the open-pollinated individuals of the parent variety.

In table 1 the results are shown of progeny tests on 34 plants selected for their resistance to wilt out of large populations of alfalfa of widely different types.[6] The nonhardy class is represented by the Spanish-Moroccan group, the widely grown American varieties by selections out of Kansas, Montana, South Dakota, and Utah Common,

[6] The authors are indebted to L. F. Graber for the initial seed stocks of a number of these lots.

and the hardier types by Grimm, Ladak, Hardistan, and the Turkistans. These plants were selfed and their offspring inoculated with the wilt organism. There are three progenies containing no resistant individuals, a result, probably, of the severity of the test. The average proportion of wilt-resistant offspring in the entire group is only about 35 percent. This result is not due to the behavior of the strains from any particular variety or class of varieties. Rather, the explanation is the occurrence of families throughout the whole series which contain very few resistant plants, together with the variable composition of the remaining progenies. If the 14 families containing one-fourth or fewer resistant segregates are eliminated the average resistance of the rest is found to be nearly 60 percent. Four plants in the group gave over 75 percent resistant offspring. These results are of considerable significance for the light they throw on the methods most likely to lead to success in breeding wilt-resistant varieties. In making selections for wilt resistance, individuals should be sought which will not only withstand the disease themselves but which will also be capable of transmitting resistance to a large proportion of their offspring. It is evident from the performance of these 34 families that selected resistant individuals differ greatly in their ability to transmit resistance, and that this ability can be determined only by a progeny test.

TABLE 1.—*Progeny tests with self-pollinated resistant selections from alfalfas of diverse origin*

Pedigree	Stock designation	Source	Plants	Resistant plants		
				Class 1	Class 2	Classes 1 and 2
			Number	*Percent*	*Percent*	*Percent*
1-33-101	F.P.I. 89904	Portugal	44	9.1	15.9	25.0
1-33-102	F.P.I. 89630	Spain	62	11.3	16.1	27.4
1-33-103	F.P.I. 89631	do	51	.0	.0	.0
1-33-104	F.P.I. 89631	do	61	.0	14.7	14.7
1-33-105	F.P.I. 89022	Chile	46	17.4	26.1	43.5
1-33-106	F.P.I. 89043	Manchuria	59	42.4	27.1	69.5
1-33-107	F.P.I. 89854	Spain	37	24.3	16.2	40.5
1-33-108	F.P.I. 89897	do	57	21.0	38.6	59.6
1-33-109	F.P.I. 011701	Morocco	64	.0	.0	.0
1-33-110	F.P.I. 011706	do	49	2.0	6.1	8.1
1-33-111	F.P.I. 84371	Turkistan	35	20.0	37.1	57.1
1-33-112	F.P.I. 84371	do	55	.0	1.8	1.8
1-33-113	F.P.I. 84371	do	46	41.3	34.8	76.1
1-33-114	F.P.I. 84371	do	59	1.7	10.2	11.9
1-33-115	F.P.I. 84371	do	70	21.4	38.6	60.0
1-33-116	F.P.I. 84371	do	54	44.4	33.3	77.7
1-33-117	1356	do	45	2.2	.0	2.2
1-33-118	1356	do	63	.0	1.6	1.6
1-33-119	2	do	71	46.5	25.3	71.8
1-33-120	F.P.I. 84443	do	52	.0	1.9	1.9
1-33-121	Hardistan	Nebraska	40	7.5	12.5	20.0
1-33-122	989	Turkistan	61	.0	16.4	16.4
1-33-123	989	do	55	9.1	36.4	45.5
1-33-124	Hardistan	Nebraska	58	6.9	15.5	22.4
1-33-125	989	Turkistan	59	15.2	44.1	59.3
1-33-127	1355	Grimm	46	.0	.0	.0
1-33-128	1356	Turkistan	68	19.1	30.9	50.0
1-33-129	875	South Dakota	23	13.0	30.4	43.4
1-33-130	1173	do	64	65.6	15.6	81.2
1-33-131	1334	Ladak	37	48.6	32.4	81.0
1-33-132	1300	Montana	45	51.1	17.8	68.9
1-33-133	1300	do	56	12.5	39.3	51.8
1-33-134	Engle	Kansas	53	43.4	30.2	73.6
1-33-135	1158	Utah	38	13.2	28.9	42.1

A series of hybrids studied involved as one parent a highly resistant plant (J3–13–2) of Turkistan origin which on self-pollination gave 21 healthy, 8 slightly diseased, and no badly diseased or dead offspring. This individual was crossed with two unrelated Grimm plants both of which on self-pollination gave entirely susceptible progenies. The F_1 hybrids were not tested for their reaction to wilt. The F_2 family from one of them, however, contained 2 class 1, 9 class 2, 95 class 3, and 53 class 4 plants. In other words, only about 7 percent of the F_2 segregates showed well-marked resistance. The other F_2 family showed 1 class 1, 11 class 2, 100 class 3, and 35 class 4 plants. Again, over 90 percent of the plants were more or less highly susceptible to the disease. There were, however, a few wilt-resistant segregates.

Another cross between an untested Grimm plant and (J3–13–2) gave no wilt-resistant F_2 plants in a population of 53.

The behavior of an F_2 population from a hybrid between the same resistant Turkistan parent (J3–13–2) and a plant of the Hairy Peruvian variety stands in marked contrast with these foregoing results. The reaction to wilt of the Hairy Peruvian individual used is not certainly known as it was not inoculated and no selfed seed was obtained. Judging, however, from the frequency with which resistant plants are found in this variety, the chances are roughly 1,000 to 1 that it was susceptible. Fifty-eight percent of the F_2 plants from this cross were resistant. The 171 plants in the family were classified as follows: 33 class 1, 66 class 2, 60 class 3, and 12 class 4. The progeny contained many large and vigorous plants which, by their habit of growth and pubescence, exhibited clear evidence of Hairy Peruvian ancestry. Among 117 F_2 plants from a similar but distinct cross involving these same varieties 18 percent resistant individuals were found. A cross between the resistant Turkistan plant, J3–13–2, and an untested, but probably susceptible, *Medicago falcata* plant gave 54 percent wilt-resistant segregates in F_2. The distribution was 13 class 1, 25 class 2, 17 class 3, and 15 class 4 plants. In this family the plant type, in general, was very different from that in the Hairy Peruvian cross, the individuals being small and semiprostrate in their habit.

The wide divergence in size and form of the plants in these two progenies from crosses of a common resistant Turkistan plant with a Hairy Peruvian individual, on the one hand, and with a *Medicago falcata* individual on the other, associated as it is with nearly equal percentages of resistant segregates, suggests that there is little if any relationship between the gross morphology of the alfalfa plant and its reaction to the wilt organism. The evidence from these two latter crosses does not support the view expressed by Peltier and Schroeder [7] that differences in resistance and susceptibility are due mainly to variations in the morphology of the plant. Attention should be called to the fact that Hairy Peruvian is a nonhardy alfalfa, whereas *Medicago falcata*, in general, can endure considerable cold. It is to be expected, consequently, that the two hybrid families would differ considerably in this respect. If they do, wilt resistance is probably not necessarily associated with winter hardiness.

[7] PELTIER, G. L., and SCHROEDER, F. R. THE NATURE OF RESISTANCE IN ALFALFA TO WILT (APLANOBACTER INSIDIOSUM L. MC.). Nebr. Agr. Expt. Sta. Research Bull. 63, 28 pp., illus. 1932.

SUMMARY AND CONCLUSIONS

A preliminary study was made of the inheritance of resistance to bacterial wilt in alfalfa. The main conclusions to which the results seem to point are as follows:

Resistance to bacterial wilt in alfalfa behaves in inheritance as an intergrading character and probably rests upon a complex genetic basis. A factorial interpretation is at present impossible.

Wilt-resistant plants may differ markedly in composition with respect to the genes governing resistance and susceptibility, as shown by the widely divergent proportions of resistant offspring obtained on selfing such individuals. The same appears to be true of wilt-susceptible plants.

The Hardistan variety comprises a diverse array of plants which on self-pollination yield families varying in number of resistant plants from 0 to 100 percent under the conditions of the test.

A study of the behavior of 95 once-selfed Hardistan families suggests that there may be a small inverse correlation between seed production and wilt resistance. The apparent relationship, however, is complex and the data are by no means conclusive.

Wilt resistance appears to bear no relationship to the external form of the plant or to winter hardiness. If this conclusion is confirmed there would seem to be no insuperable obstacle to the incorporation of wilt resistance into any of the diverse regional types of alfalfa now in use.

Insofar as it may be judged from the small sample of plants tested, Grimm alfalfa appears to be of a genetic composition, with respect to the factors conditioning resistance and susceptibility, which permits relatively few wilt-resistant segregates in crosses with highly resistant Turkistans. On the other hand, two similar matings of the Turkistans with Hairy Peruvian gave in one instance 58 percent and in the other 18 percent resistant offspring. A single cross of a resistant Turkistan with a *Medicago falcata* plant gave 54 percent resistant segregates.

INTERRELATION BETWEEN CULTURAL TREATMENT OF PECAN TREES, THE SIZE AND DEGREE OF FILLING OF THE NUTS, AND THE COMPOSITION OF KERNELS [1]

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INTRODUCTION

Only very meager data are available on the interrelation between cultural treatments, size of nuts, degree of filling, and composition of kernels in pecans. Marshall and Ruprecht (*5*) [3] compared the composition of pecan kernels from fertilized and from unfertilized trees and found very little difference in the results of the two treatments, although they state that an increase in the percentage content of oil was invariably associated with a decrease in the percentage content of protein. Skinner (*7*), reporting on the effect of various fertilizers on pecans, states that fertilizers slightly increased the size and quality of the nuts and that fertilizers highest in nitrogen produced nuts relatively better filled than did those low in nitrogen. He also states that the protein content of pecan kernels is markedly influenced by fertilizers, differences of 3 to 4 percent in protein content of the kernels having been observed in nuts from trees that received fertilizers relatively high in nitrogen as compared with those from trees that received less nitrogen or no fertilizer at all. Later, Fowler (*2*) reported that the highest percentage content of both oil and protein was found in nuts obtained from trees receiving fertilizer of an 8–8–3 [4] composition as compared with nuts from similar trees receiving equal amounts of either a 3–8–3 or a 5–8–3 fertilizer.

All workers mentioned above report that fertilizer mixtures applied to pecan trees affect the size of the nuts produced and the degree to which they are filled. It is conceivable that the analysis of nuts sampled without previous adequate separation according to size and the degree of filling would not present a true picture of the effects of fertilizer or other cultural treatment on the composition of the kernels. If marked differences in composition occur between nuts of different sizes or degrees of filling, then unless nuts of similar size and degree of filling from the different treatments are compared, the effect of various cultural treatments on composition might be masked. The effect of fertilizer or other cultural treatment on the composition of the pecan should be further investigated.

The pecan kernel is a seed consisting of a matured embryo and its cotyledons, with their stored food. Data are available showing that the composition of seeds, particularly cereals, can be consistently and significantly modified by cultural treatment and that the composition

[1] Received for publication Mar. 30, 1934; issued October, 1934.

[2] The writers wish to express their appreciation to all those who have helped in this work; especially to F. N. Dodge and N. H. Loomis, of the Division of Fruit and Vegetable Crops and Diseases, who have assisted in securing the records of crop yields and physical character of the nuts, and to the owners of the orchards in which this work was done.

[3] Reference is made by number (italic) to Literature Cited, p. 660.

[4] All fertilizer formulas are stated in the following order: N–P–K.

of the fleshy portions of fruits, such as apples and peaches, can also be affected to a certain extent by the same means.

In the fall of 1931 material was available for a study of all these factors and problems. Although the growing season that year was extremely dry, causing the nuts generally to be poorly filled, the results of such a study were expected to be of value, not only for recording and interpreting the effects of cultural treatments, but possibly also in providing a basis for the establishment of grades in the marketing of nuts.

EXPERIMENTAL PROCEDURE

The nuts for this study were harvested during October and November 1931 from four orchards located within 11 miles of Albany, Ga., where experiments are being conducted by the United States Department of Agriculture. A description of these orchards follows:

ORCHARD 1

The trees in orchard 1 are of the Stuart variety, planted in 1915, and at the beginning of the experiment were in average vegetative condition. Three treatments have been applied in this orchard since the winter of 1929–30, as follows: (1) Check, unpruned and unfertilized; (2) pruned in the dormant season; and (3) pruned in the dormant season and 10 pounds of sodium nitrate per tree applied in the spring of 1930 and 15 pounds of ammonium sulphate in March 1931.

ORCHARD 2[3]

The trees in orchard 2 are of the Stuart variety, planted in 1914, and at the beginning of the experiment were in rather poor vegetative condition, being considerably less vigorous than the Stuart trees of orchard 1. Five treatments were applied in this orchard in the spring of 1931. All the plots received fertilizer of a 5–9–3 mixture: Plot 1 received 200 pounds per tree, all applied in March; plot 2, 100 pounds per tree, all applied in March; plot 3, 100 pounds per tree, of which one-half of the nitrogen and all of the phosphorus and potash were applied in March and the remainder of the nitrogen in June; plot 4, 100 pounds per tree, of which one-half of the nitrogen was applied in March, the remainder in June, and the phosphorus and potash in October; plot 5, 15 pounds per tree, all applied in March.

ORCHARD 3

The trees in orchard 3 are of the Pabst variety, planted about 1900, and at the beginning of the experiment were in rather poor vegetative condition, with the longest terminal shoots averaging less than 6 inches. Eight treatments have been applied in this orchard. The trees in rows A and B received no treatment except that every alternate tree was pruned in the winter of 1929–30; the trees in rows C and D each received an application of 25 pounds of nitrate of soda in the fall of 1929, and every alternate tree was pruned in the winter of 1929–30; the trees in rows E and F each received in the spring of 1930 an application of 25 pounds of sodium nitrate and in the spring of 1931 an application of 30 pounds of ammonium sulphate, and every alternate tree was pruned each winter previous to the fertilizer application; the

[3] The fertilizer work in this orchard was carried on in cooperation with the Bureau of Chemistry and Soils, U.S. Department of Agriculture.

trees in rows G and H received no fertilizer, but every alternate tree was pruned in the winters of 1929–30 and 1930–31. The groups of trees receiving the different treatments were separated from one another by guard rows.

ORCHARD 4 [6]

The trees in orchard 4 are of the Schley variety, planted in 1921, and at the beginning of the experiment were in fairly good vegetative condition. In this orchard, since the spring of 1926, a different treatment has been applied to each of three rows of 23 trees each, separated by guard rows. Each tree in row A received 20 pounds of a 3–8–3 fertilizer mixture in 1926; since then the yearly fertilizer applications were gradually increased as the tree tops became larger, until the spring of 1931, when each tree received 35 pounds. The trees in rows B and C received the same weight of fertilizer each year as the trees in row A, but the formula of the fertilizer mixture for row B was 5–8–3 and for row C, 8–8–3.

SAMPLING PROCEDURE AND METHODS OF ANALYSIS

The nuts from these orchards were harvested when mature and sized according to diameter, into grades of one-sixteenth of an inch, as soon as possible after harvest. Aliquot samples of each size were secured and stored for periods ranging from 4 to 8 weeks in a dry, unheated building until cracking tests could be made and the kernels classified in regard to degree of filling. The kernels were separated into four classes according to the estimated percentage of filling: 0 to 50 percent filled; 50 to 75 percent filled; 75 to 90 percent filled; and 90 to 100 percent filled. Because the lowest class lacked commercial value and the highest class included too few nuts, only the two medium classes were saved for the analyses, the results of which are presented in this paper.

The classification of the kernels according to the degree of filling was based entirely upon their plumpness and freedom from wrinkles and upon the degree of hollowness in the halves of the kernels.

After classification according to the degree of filling, certain size combinations were made. From orchard 2 all the kernels of all sizes falling into the 50-to-75-percent-filled class were combined, as were those of all sizes falling into the 75-to-90-percent-filled class. Two size combinations were made of the kernels from all other orchards, the kernels from nuts falling into the 14/16 or 15/16-inch sizes and larger being combined into one class, as noted in the tables, and the remaining kernels from the smaller nuts into a second class. After being thus separated and recombined, the samples of kernels were mixed and portions sufficient for the analyses were placed immediately in pint Mason jars and stored at a temperature of approximately 0° C. until the analyses could be made.

PREPARATION OF SAMPLES FOR ANALYSIS

After being removed from storage the kernels were sliced on an inverted jack plane upon which was mounted a wooden slide supporting a hopper. This method provided a means of obtaining slices approximately 50μ in thickness without entailing the loss of undetermined amounts of oil as when the kernels are ground in a food chopper.

[6] See footnote 5.

Moisture Determination

The sliced samples were well mixed and approximately 10 g were placed in weighing bottles for the determination of moisture. In this determination the weighed samples were dried for 5 hours at 65° C. and under 12.5 inches of vacuum, and again weighed. The resulting loss in weight represents the moisture content.

Oil Determination

The percentage of oil was determined by a slightly modified form of a method outlined by Lewis (*4*). This method has greater rapidity than the usual ether-extraction method with little or no loss in accuracy. In the modified method used, 4 g of the sliced kernels were placed in a 9-g 50-percent Babcock-type cream-test bottle. To this 4-g sample was added 35 cc of a sulphuric acid solution containing 1.5 parts of concentrated acid to 1 part of water. The bottles were then placed in an oven for at least 2 hours at a temperature of 65° C. During this time they were frequently shaken to aid digestion. The bottles were then placed in a regulation Babcock centrifuge and whirled for 5 minutes at a speed of about 900 revolutions per minute. Enough of the acid solution was then added to fill the bottles to the base of the neck, after which they were again centrifuged, this time for 3 minutes. Again acid solution was added to each bottle in sufficient quantity to fill about two-thirds of the neck, after which the bottles were given a final centrifuging for 1 minute and then returned to the oven for at least 15 minutes at a temperature of 55°. By the aid of calipers the height of the oil column was then read in terms of spaces occupied. It was found by Lewis that 1 g of pecan oil at a temperature of 55° occupied 11.18 spaces in the neck of a cream-test bottle of this size; consequently, it was an easy matter to calculate the percentage of oil in the sample. All determinations for oil were run in duplicate, and calculation of the percentage content of oil was based on the average of the two readings unless deviations greater than one-half space occurred, in which case additional samples were tested.

Nitrogen Determination

The nitrogen content was obtained from the dry samples resulting from the determination of moisture and was estimated by the Gunning method modified to include the nitrogen of nitrates (*1*), even though separate determinations for organic nitrogen alone showed that no measurable quantities of nitrate nitrogen were present. The percentages of nitrogen were multiplied by the factor 5.30 for conversion into terms of protein. The use of this factor is based on the work of Jones (*3*), who found that the commonly accepted factor 6.25 was not accurate when applied to oily seeds and nuts and suggested the foregoing factor as giving a more accurate estimate of the protein content. Although Jones did not work with pecans, the factor that he suggests is considered applicable, because this nut is closely related to the black walnut and butternut, the species with which he worked.

Carbohydrates

The carbohydrates were determined by an arbitrary method of acid hydrolysis designed to give results comparable among samples. In the procedure used, accurately weighed 5-g samples of the sliced

nut meats were placed in 500-cc Erlenmeyer flasks to which 225 cc of a 2.5 percent (by weight) aqueous solution of sulphuric acid was added, and heated for 2½ hours over a boiling water bath. The samples were immediately filtered by suction and the filtrate allowed to cool. Sufficient concentrated sodium hydroxide solution was then added to make the samples just acid to phenolphthalein. Approximately one-half of a gram of neutral lead acetate was added to each of the filtrates, which were well shaken and again filtered. The addition of 10 cc of a saturated solution of disodium phosphate to each filtrate, to precipitate the excess lead, and refiltering, completed the preparation for making to volume in a 500-cc volumetric flask. The invert sugars were determined on 50-cc aliquots by the use of Soxhlet's modification of Fehling's solution, and the amounts and conditions of heating were those prescribed by Munson and Walker (*1*). The amount of cuprous oxide thus obtained was determined by the method of iodometric titration outlined by Shaffer and Hartmann (*6*).

The fact that this procedure gave substantially the same results from duplicate samples and that hydrolysis on the water bath for 2½ hours with a 12 percent (by weight) aqueous solution of sulphuric acid gave no greater yield of invert sugars indicates that the data obtained are fairly accurate and dependable.

The carbohydrates determined by the method just described were estimated by the use of Munson and Walker's table (*1*). The percentage carbohydrate content of the kernels was calculated on the basis of the weight of glucose resulting from the acid hydrolysis.

All data are presented on the basis of the weight of freshly sliced samples of well-cured kernels.

PRESENTATION OF DATA

In the presentation of the data, each orchard is considered separately. The data for each orchard have been divided into three parts, all in relation to the influence of the cultural treatments used: (1) Size of nuts produced; (2) specific gravity, weight of kernel, and degree of filling of the nuts in relation to their size; and (3) composition of the kernels in relation to size and degree of filling of the nuts.

ORCHARD 1

In orchard 1 the effects of pruning and nitrogen applications on tree growth and nut production are being studied.

Size of Nuts

Table 1 shows that the methods of culture used in this Stuart orchard influenced the percentage of nuts of certain sizes in the total 1931 nut crop. Pruning appreciably increased the percentage of nuts sixteen-sixteenths of an inch or larger in diameter, and reduced the percentage of small nuts as compared with those produced by the check trees. Applications of nitrogen in addition to pruning failed to increase further the percentage of nuts of the larger sizes in the total nut crop, but it did increase the percentage of nuts thirteen-sixteenths of an inch or less in diameter. This apparently was because the application of nitrogen increased the set of nuts, some of which were borne on weak shoots and would probably have dropped without this stimulation.

TABLE 1.—*Influence of cultural treatment on percentage[1] of Stuart pecan nuts of various sizes, in orchard 1, crop of 1931*

Treatment	Nuts of indicated diameter					
	11/16 inch	12/16 inch	13/16 inch	14/16 inch	15/16 inch	16/16 inch up
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	0.0	[2]	0.2	6.6	56.0	37.2
Pruned	.0	[2]	.1	1.6	48.8	49.5
Pruned plus nitrogen	.2	0.2	.9	4.8	48.8	45.1

[1] Percentage of total crop.

[2] Less than 0.1 percent of total crop.

SPECIFIC GRAVITY, WEIGHT OF KERNEL, AND DEGREE OF FILLING

The data presented in table 2 show that the practice of pruning, with or without the application of nitrogen, materially influenced the character of the nuts produced by the Stuart pecan trees used. In this orchard, pruning, alone or with nitrogen applications, increased not only the specific gravity of the whole nuts, but also the average weight of the kernel and the degree to which the nuts were filled. The whole nuts from the pruned trees had a higher specific gravity and heavier kernels than nuts of the same size produced by the check trees. Likewise, pruning, as compared with the checks, increased the percentage of the nuts which were 75 to 90 percent filled. Applications of nitrogen in addition to the pruning of the trees did not result in any appreciable differences in the character of the nuts produced as compared with those borne on the trees which were only pruned. It should be pointed out that the size of the nuts from all treatments had not only an important relation to the average weight of the kernel but to the degree to which the nuts were filled. In general, the nuts of the small sizes produced by all treatments were more poorly filled than those of the large sizes. In all cases, nuts fifteen-sixteenths of an inch or more in diameter had a much smaller percentage 0 to 50 percent filled than nuts fourteen-sixteenths of an inch or less in diameter.

TABLE 2.—*Influence of cultural treatment on average specific gravity, weight of kernel, and degree of filling of Stuart pecan nuts of various sizes, in orchard 1, crop of 1931*

Treatment	Diameter of nuts	Specific gravity of whole nuts	Weight of kernels	Nuts filled—		
				0 to 50 percent	50 to 75 percent	75 to 90 percent
	Inch		*Grams*	*Percent*	*Percent*	*Percent*
Check	13/16	0.682	1.32	0.00	33.33	66.66
	14/16	.606	1.93	20.00	46.66	33.33
	15/16	.655	2.69	12.14	46.43	41.43
	16/16-up	.690	3.33	8.62	38.79	52.59
Pruned	12/16	.702	3.90	.00	20.79	79.21
	13/16	.617	1.39	33.33	33.33	33.33
	14/16	.723	2.22	8.33	8.33	83.34
	15/16	.687	2.94	10.61	25.00	64.39
	16/16-up	.707	3.66	4.35	22.61	73.04
Pruned plus nitrogen	11/16	.712	4.03	1.92	17.31	80.77
	12/16	.710	1.33	16.00	16.00	68.00
	13/16	.716	1.68	12.68	25.35	61.97
	14/16	.720	2.22	24.17	24.73	51.10
	15/16	.717	3.08	4.32	15.83	79.85
	16/16-up	.684	3.64	2.73	29.09	68.18
		.701	4.04	2.97	23.76	73.27

Composition of Kernels

A study of table 3 showing the data for the Stuart nuts from orchard 1 brings out several interesting points. A comparison between samples of kernels, comparable as to size and treatment but of different degrees of filling, shows consistent differences in percentage composition. Without exception, the 75 to 90 percent filled nut samples show a higher percentage content of oil and a lower percentage content of protein, carbohydrates, water, and undetermined constituents than the 50 to 75 percent filled samples. Likewise, it is consistently true that in all comparable samples, as the percentage content of oil increases the percentage content of protein, carbohydrates, water, and undetermined constituents decreases. No such clear-cut and consistent differences are evident, however, in a comparison between either sizes or treatments. The samples of the smaller sized nuts show neither a consistently higher nor a consistently lower percentage content of oil, protein, carbohydrates, water, or undetermined constituents than do the samples of the larger sized nuts. Moreover, the differences between comparable samples from the three cultural treatments given are neither of sufficient magnitude nor consistency to indicate any effect of the treatment on composition, even though the plot receiving the nitrogenous fertilizers shows a slightly greater percentage content of oil and a slightly smaller percentage content of protein than do the nuts from the other two plots. These differences are small and may be due to the method of sampling as readily as to the effect of the treatments given.

Table 3.—*Influence of cultural treatment on composition of kernels in Stuart pecan nuts of various sizes and degrees of filling, in orchard 1, crop of 1931*

Treatment	Diameter of nuts	Composition of kernels		
		Constituent	In nuts filled—	
			50 to 75 percent	75 to 90 percent
	Inch		*Percent* [1]	*Percent* [1]
Check	15/16 and oversize	Oil	58.69	61.49
		Protein	18.05	17.11
		Carbohydrates	9.87	9.44
		Water	2.41	2.20
		Undetermined	10.98	9.76
	13/16 to 15/16	Oil	57.03	65.41
		Protein	17.05	16.07
		Carbohydrates	10.98	7.59
		Water	3.13	2.17
		Undetermined	11.81	8.76
Pruned	15/16 and oversize	Oil	58.70	63.74
		Protein	17.56	16.83
		Carbohydrates	10.67	8.97
		Water	2.57	2.34
		Undetermined	10.50	8.12
	13/16 to 15/16	Oil	57.02	62.62
		Protein	17.56	16.75
		Carbohydrates	10.86	9.11
		Water	2.58	2.14
		Undetermined	11.98	9.38
Pruned plus nitrogen	15/16 and oversize	Oil	59.26	64.28
		Protein	16.82	15.62
		Carbohydrates	9.75	8.01
		Water	2.95	2.46
		Undetermined	11.22	9.63
	19/16 to 15/16	Oil	64.84	65.97
		Protein	14.88	14.75
		Carbohydrates	7.75	7.59
		Water	2.51	2.37
		Undetermined	10.02	9.32

[1] Percentage of cured weight.

ORCHARD 2

In orchard 2 a study is being made of the effects of varying amounts and times of application of fertilizers on tree growth and nut production.

SIZE OF NUTS

Fertilizers applied for the first time in the spring of 1931 affected materially the size of the Stuart nuts produced in this orchard the same year, as shown by the data of table 4. Except on plot 4, the heavy applications of fertilizer made to the trees in this experiment significantly increased the percentage of nuts that were fifteen-sixteenths of an inch or more in diameter. Plot 4 produced a higher percentage of small nuts, i.e., fourteen-sixteenths of an inch or less in diameter, than did the check plot 5. It is not understood why equivalent amounts of nitrogen applied at the same time to plots 3 and 4 resulted in such differences in the percentage of large nuts produced, unless the additional phosphorus and potassium applied to plot 3 in March counteracted the stimulatory effects of the nitrogen, or most probably these results were due to differences in soil conditions between the two plots. The data in table 4 should not be construed as indicating the results that may be expected to follow fertilizer applications, as the data are for 1 year only, and during that year occurred one of the severest droughts ever experienced in the pecan-growing district of Georgia. The significant fact brought out by the data is that the percentage of nuts of the large sizes varied between treatments.

TABLE 4.—*Influence of amount of fertilizer and of time of application on percentage* [1] *of Stuart pecan nuts of various sizes, in orchard 2, crop of 1931*

Plot no.	Fertilizer applied [2]		Nuts of indicated diameter				
	Per tree	Month	13/16 inch	13/16 inch	14/16 inch	15/16 inch	16/16 inch–up
	Pounds		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	200	March	0.1	0.6	17.7	70.8	10.8
2	100	do	.1	.5	14.3	69.9	15.2
3	100	½ N in March; ½ N in June; all K and P in March	.2	.7	14.0	67.2	17.9
4	100	½ N in March; ½ N in June; all K and P in October	.2	1.7	31.3	61.6	5.1
5	15	March	.2	1.7	27.4	58.6	12.1

[1] Percentage of total crop. [2] Fertilizer mixture: 5 N-9 P-3 K.

SPECIFIC GRAVITY, WEIGHT OF KERNEL, AND DEGREE OF FILLING

In table 5 are the data for specific gravity, average weight of kernel, and degree of filling for the nuts produced by different fertilizer treatments. These data show that heavy applications of fertilizer in plots 1 to 4, inclusive, reduced the specific gravity of the whole nuts and the average weight of the kernels of the small nuts, when compared with plot 5, which received a small amount of fertilizer. Furthermore, the specific gravity of the whole nuts, in general, was in relation to the diameter of the nuts; i.e., the small nuts were less well filled than those of the large sizes. The degree to which the nuts were filled in this experiment for the crop year 1931 was very poor. Under the

severe drought conditions trees receiving fertilizers produced nuts that were less well filled than those of the check trees. Plot 5, which received only a small amount of fertilizer per tree, produced a significantly higher percentage of nuts that were 75 to 90 percent filled than plots 1 to 4, which received much heavier applications of fertilizer. This condition was probably due to the effect of the fertilizer in stimulating leaf production in the spring and to the occurrence of the drought, which caused the fertilized trees having a large leaf area to suffer more from water shortage than the trees that received less fertilizer and consequently had a smaller leaf area.

TABLE 5.—*Influence of amount of fertilizer and of time of application on average specific gravity, weight of kernel, and degree of filling of Stuart pecan nuts, in orchard 2, crop of 1931*

Plot no.	Fertilizer applied [1]		Diameter of nuts	Specific gravity of whole nuts	Weight of kernels	Nuts filled—		
	Per tree	Month				0 to 50 percent	50 to 75 percent	75 to 90 percent
	Lb.		*Inch*			*Percent*	*Percent*	*Percent*
			13/16	0.427	0.34	92.3	7.7	0.0
			14/16	.581	1.94	18.9	66.1	15.0
1	200	March	15/16	.626	2.59	1.4	85.7	12.9
			16/16	.634	2.98	1.7	91.6	6.7
			16/16-up	.638	3.26	1.8	92.6	5.6
			13/16	.453	.54	71.4	28.6	.0
			14/16	.587	1.93	23.2	66.4	10.4
2	100	do	15/16	.631	2.67	2.1	92.9	5.0
			16/16	.630	2.90	1.6	96.7	1.7
			16/16-up	.640	3.25	.9	91.7	7.4
			13/16	.470	.56	77.4	22.6	.0
			14/16	.580	1.66	31.7	63.4	4.9
3	100	½ N in March; ½ N in June; all K and P in March	15/16	.617	2.39	10.3	86.8	2.9
			16/16	.614	2.67	.8	98.4	.8
			16/16-up	.637	3.27	4.7	93.4	1.9
			13/16	.522	.99	51.4	41.9	6.7
			14/16	.627	2.18	10.1	85.7	4.2
4	100	½ N in March; ½ N in June; all K and P in October	15/16	.646	2.69	3.4	93.1	3.5
			16/16	.624	2.77	6.3	87 3	6.4
			16/16-up	.646	3.24	2.8	92.5	4.7
			13/16	.551	1.25	27.9	50.0	22.1
			14/16	.656	2.16	4.8	27.9	67.3
5	15	March	15/16	.650	2.59	2.8	34.8	62.4
			16/16	.634	2.86	2.4	63.4	34.2
			16/16-up	.633	3.16	4.8	73.3	21.9

[1] Fertilizer mixture: 5N-9P-3K.

COMPOSITION OF KERNELS

The data from analyses of Stuart nuts from orchard 2 shown in table 6 indicate a relationship between grades, sizes, and treatments similar to that shown for orchard 1 in table 3, despite the wide variation in treatment of the two orchards. One additional point of interest is to be observed. Both sets of data deal with nuts of the Stuart variety from trees of approximately the same age. The data in table 3 on the protein content of nuts from trees in orchard 1, which are of a much younger physiological age, due to the better care and to the nitrogenous fertilizers that they had received in the past, show a considerably higher content than that of the nuts from orchard 2, the data for which are presented in table 6. To what this condition is due cannot be determined definitely from the data available, although again there is an indication that the treatment may have had some influence on the composition of the nuts. However, this conclusion is not substantiated by a comparison of the nut samples from different treatments in the same orchard.

TABLE 6.—*Influence of amount of fertilizer and of time of application on composition of kernels in Stuart pecan nuts of various degrees of filling, in orchard 2, crop of 1931*

[All sizes of nuts were combined in sampling from this orchard]

Plot no.	Fertilizer[1] applied		Composition of kernels		
	Per tree	Month	Constituent	In nuts filled— 50 to 75 percent	In nuts filled— 75 to 90 percent
	Lbs.			*Percent*[2]	*Percent*[2]
1	200	March	Oil	60.38	63.73
			Protein	13.81	13.32
			Carbohydrates	10.88	9.52
			Water	3.19	2.95
			Undetermined	11.74	10.48
2	100	do	Oil	58.70	64.85
			Protein	13.68	13.48
			Carbohydrates	10.14	9.12
			Water	3.68	3.32
			Undetermined	13.80	9.23
3	100	½ N in March; ½ N in June; all K and P in March	Oil	58.14	64.84
			Protein	14.76	11.84
			Carbohydrates	10.26	8.18
			Water	3.98	3.26
			Undetermined	12.86	11.88
4	100	½ N in March; ½ N in June; all K and P in October	Oil	59.53	66.25
			Protein	14.86	13.10
			Carbohydrates	9.36	7.86
			Water	5.32	3.79
			Undetermined	10.93	9.00
5	15	March	Oil	59.82	64.29
			Protein	13.25	12.26
			Carbohydrates	9.90	8.98
			Water	3.47	2.80
			Undetermined	13.56	11.67

[1] Fertilizer mixture: 5 N-9 P-3 K.

[2] Percentage of cured weight.

ORCHARD 3

In orchard 3 the practice of pruning, with and without nitrogen applications, has been studied in relation to tree growth and nut production.

TABLE 7.—*Influence of cultural treatment on percentage of Pabst pecan nuts of various sizes, in orchard 3, crop of 1931*

Row[1] and treatment	Nuts of indicated diameter					
	11/16 inch	12/16 inch	13/16 inch	14/16 inch	15/16 inch	16/16 inch.–up
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Rows E and F,[2] unpruned; nitrogen fertilizer	0.1	3.1	25.2	56.5	14.7	0.4
Rows E and F,[2] pruned; nitrogen fertilizer	.2	2.6	21.2	51.4	22.9	1.7
Rows G and H, unpruned; unfertilized	.1	3.1	21.3	51.1	23.1	1.3
Rows G and H, pruned; unfertilized	.1	1.7	16.6	50.6	29.2	1.8

[1] Since the data for rows A to D show the same trend as the data given, they are not included in this table.

[2] Each tree in these rows received 25 pounds of sodium nitrate in early spring of 1930 and 30 pounds of ammonium sulphate in early spring of 1931.

SIZE OF NUTS

The data of table 7 show that pruning increased the size of the nuts produced as compared with those borne by similar unpruned trees, and that applications of nitrogen to the trees, regardless of whether they were pruned or not, had a tendency to reduce the percentage of nuts that were fifteen-sixteenths of an inch or more in diameter. The most probable explanation for these results is that the heavier crops and larger leaf area of the fertilized trees made greater demands for water during the drought and reduced the size of the nuts produced

as compared with those borne by similar unfertilized trees. The important fact brought out by these data is that both pruning and fertilization, singly and in combination, did affect the percentage of nuts that were fifteen-sixteenths of an inch or more in diameter.

Specific Gravity, Weight of Kernel, and Degree of Filling

In this experiment with Pabst trees both pruning and nitrogen applications, separately or in combinations, influenced significantly the character of the nuts produced. The data given in table 8 show the effects of these treatments. The outstanding effect was that resulting from applications of nitrogen, which appreciably increased the specific gravity of the whole nuts, the average weight of the kernels, and the degree to which the nuts were filled, as compared with nuts borne by similar unfertilized trees. Pruning alone had little or no effect on these nut characters, but when nitrogen was applied in addition to pruning, nuts of the highest quality, from all standpoints, were produced. In this experiment the differences in the quality of the nuts produced by the various treatments are so wide as to warrant no further discussion. However, the relation between size of nuts and the degree to which they were filled should be pointed out. In general, the small nuts, thirteen-sixteenths of an inch or smaller in diameter, were filled to a much lesser degree than those of larger diameter.

Table 8.—*Influence of cultural treatment on average specific gravity, weight of kernel, and degree of filling of Pabst pecan nuts of various sizes, in orchard 3, crop of 1931*

Row [1] and treatment	Diameter of nuts	Specific gravity of whole nuts	Weight of kernels	Nuts filled—			
				0 to 50 percent	50 to 75 percent	75 to 90 percent	90 to 100 percent
	Inch		*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Rows E and F,[2] unpruned; nitrogen fertilizer	13/16	0.602	1.37	5.9	52.9	41.2	0.0
	14/16	.674	2.00	15.8	19.6	63.0	1.6
	15/16	.731	2.97	2.7	9.9	84.8	2.7
	16/16-up	.739	3.29	6.0	6.7	85.8	1.5
Rows E and F,[2] pruned; nitrogen fertilizer	13/16	.749	3.53	6.3	5.4	88.4	.0
	14/16	.798	3.93	2.8	8.3	88.9	.0
	15/16	.708	1.75	6.2	31.3	62.5	.0
	16/16-up	.713	2.15	10.9	21.8	65.6	1.7
Rows G and H, unpruned; unfertilized	13/16	.700	2.76	10.8	17.3	67.6	4.3
	14/16	.734	3.38	7.5	6.5	85.1	.9
	15/16	.743	3.81	3.3	11.0	83.5	2.2
	16/16-up	.761	4.08	3.9	4.9	91.2	.0
Rows G and H, pruned; unfertilized	13/16	.548	1.04	28.0	60.0	12.0	.0
	14/16	.638	1.76	21.7	29.9	48.4	.0
	15/16	.682	2.60	11.0	20.0	69.0	.0
	16/16-up	.706	3.11	6.8	9.4	83.8	.0
	13/16	.744	3.61	5.8	11.6	82.5	.0
	14/16	.754	3.84	3.9	9.8	86.3	.0
	15/16	.589	1.10	15.4	69.2	15.4	.0
	16/16-up	.646	1.71	23.5	30.1	46.4	.0
	13/16	.683	2.58	12.2	23.0	64.8	.0
	14/16	.727	3.31	5.3	8.9	85.8	.0
	15/16	.735	3.57	1.9	20.2	77.9	.0
	16/16-up	.745	3.83	6.9	9.2	83.9	.0

[1] Since the data for rows A to D show the same trend as the data given, they are not included in this table.
[2] Each tree in these rows received 25 pounds of sodium nitrate in early spring of 1930 and 30 pounds of ammonium sulphate in early spring of 1931.

Composition of Kernels

The data presented in table 9 show relationships similar to those previously noted in tables 3 and 6. The percentage content of oil is higher and the percentage content of protein lower than that of the Stuart nuts from both orchards 1 and 2. The other constituents show similar ranges in percentage content. The data indicate a rather remarkable uniformity in composition of all samples of nuts of comparable filling, irrespective of their size or of the treatment given. There are a few discrepancies in these data on the Pabst nuts between samples of different degrees of filling, but the discrepancies are so few and so small that the reliability of the data cannot be questioned.

Table 9.—*Influence of cultural treatment on composition of kernels in Pabst pecan nuts of various sizes and degrees of filling, in orchard 3, crop of 1931*

Row and treatment	Diameter of nuts	Composition of kernels		
		Constituent	In nuts filled—	
			50 to 75 percent	75 to 90 percent
	Inch		*Percent* [1]	*Percent* [1]
Rows A and B, pruned; unfertilized	14/16, 15/16, and oversize	Oil	68.20	70.44
		Protein	11.05	9.42
		Carbohydrates	9.21	7.81
		Water	3.49	3.36
		Undetermined	8.05	8.97
	13/16 to 13/16	Oil	66.25	71.56
		Protein	10.73	9.87
		Carbohydrates	9.17	8.80
		Water	4.02	4.00
		Undetermined	9.83	5.77
Rows A and B, unpruned; unfertilized	14/16, 15/16, and oversize	Oil	69.32	70.44
		Protein	10.67	11.38
		Carbohydrates	9.46	8.74
		Water	4.09	4.10
		Undetermined	6.46	5.34
	12/16 [2] to 13/16	Oil	61.78	65.41
		Protein	12.24	12.09
		Carbohydrates	10.66	8.67
		Water	4.18	3.78
		Undetermined	11.14	10.05
Rows C and D,[3] pruned; nitrogen fertilizer	14/16, 15/16, and oversize	Oil	66.81	70.43
		Protein	11.14	10.01
		arbohydrates	9.36	8.62
		Water	4.08	3.66
		Undetermined	8.61	7.28
	13/16 to 13/16	Oil	65.96	67.92
		Protein	11.84	11.63
		arbohydrates	8.87	9.36
		Water	3.73	4.16
		Undetermined	9.60	6.93
Rows C and D,[3] unpruned: nitrogen fertilizer	14/16, 15/16, and oversize	Oil	66.52	70.44
		Protein	10.79	9.90
		arbohydrates	9.39	8.40
		Water	3.48	3.69
		Undetermined	9.82	7.57
	13/16 to 13/16	Oil	64.85	69.32
		Protein	12.08	10.59
		arbohydrates	10.04	8.43
		Water	3.81	3.49
		Undetermined	9.22	8.17
Rows E and F,[4] pruned; fertilized	14/16, 15/16, and oversize	Oil	65.41	69.88
		Protein	10.93	10.88
		arbohydrates	9.82	8.08
		Water	3.16	2.69
		Undetermined	10.68	8.47
	13/16 to 13/16	Oil	64.84	69.60
		Protein	11.58	10.74
		arbohydrates	9.03	7.98
		Water	2.93	2.51
		Undetermined	11.62	9.17

[1] Percentage of cured weight.
[2] The sample of nuts having diameters of eleven-sixteenths inch was lost.
[3] Each tree in these rows received 25 pounds of sodium nitrate in the fall of 1929.
[4] Each tree in these rows received 25 pounds of sodium nitrate in early spring in 1930 and 30 pounds of ammonium sulphate in early spring in 1931.

TABLE 9.—*Influence of cultural treatment on composition of kernels in Pabst pecan nuts of various sizes and degrees of filling, in orchard 3, crop of 1931*—Con.

Row and treatment	Diameter of nuts	Composition of kernels		
		Constituent	In nuts filled—	
			50 to 75 percent	75 to 90 percent
	Inch		*Percent*	*Percent*
Rows E and F,[4] unpruned; fertilized	14/16, 15/16, and oversize	Oil	67.64	69.32
		Protein	10.70	10.48
		Carbohydrates	8.66	7.33
		Water	2.46	2.57
		Undetermined	10.54	10.30
	11/16 to 13/16	Oil	64.85	69.60
		Protein	11.82	11.00
		Carbohydrates	8.74	5.64
		Water	3.05	2.96
		Undetermined	11.54	10.80
Rows G and H, pruned; unfertilized	14/16, 15/16, and oversize	Oil	66.53	70.15
		Protein	11.02	10.41
		Carbohydrates	10.52	8.30
		Water	2.78	2.55
		Undetermined	9.15	8.56
	11/16 to 13/16	Oil	67.92	69.32
		Protein	10.70	10.61
		Carbohydrates	9.44	9.25
		Water	2.50	2.43
		Undetermined	9.44	8.39
Rows G and H, unpruned; unfertilized	14/16, 15/16, and oversize	Oil	65.97	69.60
		Protein	11.84	11.77
		Carbohydrates	9.32	7.74
		Water	2.86	2.58
		Undetermined	10.01	8.31
	11/16 to 13/16	Oil	66.25	68.76
		Protein	11.33	11.20
		Carbohydrates	9.57	6.84
		Water	3.26	3.04
		Undetermined	9.59	10.16

[4] Each tree in these rows received 25 pounds of sodium nitrate in early spring in 1930 and 30 pounds of ammonium sulphate in early spring in 1931.

ORCHARD 4

In orchard 4 the effects of varying amounts of nitrogen, applied in a complete fertilizer, on pecan tree growth and yield of nuts are being studied.

SIZE OF NUTS

The data of table 10 show that the size of Schley nuts produced in this experiment was in relation to the amount of nitrogen available to the trees. The trees of row A, which received the smallest quantities of nitrogen, produced the lowest percentage of nuts that were fourteen-sixteenths of an inch or larger in diameter, whereas the trees in row C, which received the largest quantities of nitrogen, produced the highest percentage of large nuts. The trees of row B hold the intermediate position between those of rows A and C, both in the amount of nitrogen applied and in the size of the nuts borne.

TABLE 10.—*Influence of amount of nitrogen on percentage* [1] *of Schley pecan nuts of certain sizes, in orchard 4, crop of 1931*

Row	Fertilizer mixture [2]	Percentage of nuts of indicated diameter					
		10/16 inch	11/16 inch	12/16 inch	13/16 inch	14/16 inch	15/16 inch
A	3-8-3	[3]	0.3	5.5	50.2	41.3	2.7
B	5-8-3	[3]	.3	6.7	41.0	46.9	5.1
C	8-8-3	[3]	.4	5.7	39.9	47.2	6.8

[1] Percentage of total crop.
[2] Fertilizer analysis stated in order of N–P–K.
[3] Less than 0.1 percent of total crop.

Specific Gravity, Weight of Kernel, and Degree of Filling

The data in table 11 show that the fertilizer treatment influences the quality of the nuts produced, as already brought out by previous data. It was shown in table 10 that the size of the nuts bore a positive relation to the amount of nitrogen applied to the trees. The data in table 11 show that in general the average specific gravity, weight of kernel, and degree of filling bear an inverse relation to the amount of nitrogen available to the trees. The trees of row A, which received the smallest amounts of nitrogen, produced nuts which, with few exceptions, had the highest specific gravity and the heaviest kernels, and also the highest percentage of nuts that were 75 to 100 percent filled; whereas the trees of row C, which received the largest amounts of nitrogen, bore nuts the two largest sizes of which were better filled than the smaller sizes, but these nuts were less well filled than those from trees receiving either of the two other treatments. In this experiment, as in the others preceding, the small nuts from all treatments were more poorly filled than the large nuts.

Table 11.—*Influence of amount of nitrogen on average specific gravity, weight of kernel, and degree of filling of Schley pecan nuts of various sizes, in orchard 3, crop of 1931*

Row	Fertilizer mixture [1]	Diameter of nuts	Specific gravity of whole nuts	Weight of kernels	Nuts filled—			
					0 to 50 percent	50 to 75 percent	75 to 90 percent	90 to 100 percent
		Inch		*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
A	3-8-3	19/64	0.544	0.70	70.0	20.0	10.0	0.0
		15/32	.558	1.27	35.3	27.3	35.4	2.0
		23/64	.633	2.31	10.6	37.1	48.2	4.1
		13/32	.662	2.84	2.7	40.8	51.0	5.5
		29/64	.692	3.27	2.2	22.6	65.7	9.5
		15/32	.685	3.44	2.3	22.3	62.3	13.1
B	5-8-3	19/64	.524	.65	75.0	6.3	18.7	.0
		15/32	.547	1.21	33.8	35.8	28.6	2.0
		23/64	.588	1.97	16.1	40.8	42.0	1.1
		13/32	.635	2.73	6.2	34.2	54.1	5.5
		29/64	.662	3.24	.7	31.3	59.7	8.2
		15/32	.664	3.37	3.4	21.0	67.2	8.4
C	8-8-3	19/64	.425	.35	72.0	24.0	4.0	.0
		15/32	.519	1.17	42.0	32.5	25.5	.0
		23/64	.575	1.91	20.0	57.7	19.4	2.9
		13/32	.629	2.72	6.0	34.7	52.0	7.3
		29/64	.700	3.30	5.8	37.0	45.6	11.6
		15/32	.701	3.77	2.4	30.6	57.3	9.7

[1] Fertilizer analysis stated in order of N–P–K.

Composition of Kernels

The data on the percentage composition of nuts of the Schley variety from the three experimental plots in orchard 4 are presented in table 12. These data indicate relationships between the constituents similar to those previously noted, even though the actual percentage values show some variation. It is to be noted, however, that the percentage content of oil is about the same or somewhat lower than that of the Pabst nuts and that the percentage content of protein is also somewhat lower, whereas the percentage content of the carbohydrates, water, and undetermined constituents is generally higher. These data thus indicate a varietal difference in the percentage content of the various constituents in comparable samples, without at the

same time altering the relationships between the constituents and the degree of filling, the size, and the cultural treatments.

But little evidence is added by these data to indicate any effect of treatment, unless the slightly higher oil content and the slightly lower protein content of the samples receiving the fertilizer mixture containing the highest percentage of nitrogen is considered of sufficient magnitude to warrant such a conclusion. These differences cannot be considered very significant, however, in the light of the fact that the three treatments mentioned have been applied each year for the past 6 years.

TABLE 12.—*Influence of amount of nitrogen on composition of kernels in Schley pecan nuts of various sizes and degrees of filling, in orchard 4, crop of 1931*

Row	Fertilizer mixture [1]	Diameter of nuts	Composition of kernels		
			Constituent	In nuts filled—	
				50 to 75 percent	75 to 90 percent
		Inch		*Percent* [2]	*Percent* [2]
A	3-8-3	14/16 and 15/16	Oil	63.17	67.09
			Protein	9.62	9.93
			Carbohydrates	11.13	10.14
			Water	5.93	4.91
			Undetermined	10.15	7.93
		10/16 to 13/16	Oil	60.37	65.68
			Protein	9.89	9.70
			Carbohydrates	11.09	10.61
			Water	8.97	5.68
			Undetermined	9.68	8.33
B	5-8-3	14/16 and 15/16	Oil	63.18	67.64
			Protein	9.04	8.29
			Carbohydrates	10.70	10.02
			Water	4.72	4.66
			Undetermined	12.36	9.39
		10/16 to 13/16	Oil	59.52	63.74
			Protein	9.98	10.06
			Carbohydrates	11.76	10.61
			Water	5.85	5.14
			Undetermined	12.89	10.45
C	8-8-3	15/16, 15/16 and oversize	Oil	68.20	71.00
			Protein	8.77	8.76
			Carbohydrates	9.36	9.03
			Water	3.19	2.73
			Undetermined	10.48	8.48
		10/16 to 13/16	Oil	64.84	69.59
			Protein	9.49	8.47
			Carbohydrates	11.99	8.17
			Water	2.68	2.49
			Undetermined	11.00	11.28

[1] Fertilizer analysis stated in order of N–P–K. [2] Percentage of cured weight.

DISCUSSION AND CONCLUSIONS

In the developing and maturing of the pecan nut there are apparently two very important periods: (1) The period of growth, during which the nuts and all their parts enlarge and which begins at blossoming and probably ends with the hardening of the shell; and (2) the period of filling the nut, which begins probably about the time the shell begins to harden and ends with the separation of the nut from the shuck. Cultural practices and seasonal conditions during the first period affect the size of the nuts, and during the second period they determine the degree to which the nuts are filled.

During the period of enlargement, food materials moving into the nuts, though probably utilized to a large extent in tissue forma-

tion, accumulate slowly. It is probably not until after the shell has hardened that carbohydrates begin to move into the nuts in large quantities, for storage in one form or another. This seasonal movement of materials modifies the size, degree of filling, and percentage or total composition of the nuts in varying degrees each year, depending upon the cultural practices used and the seasonal conditions prevailing during the two parts of the growing season.

The results of this investigation show that the cultural treatments employed in a pecan orchard materially affect the size of the nuts produced and the degree to which they are filled. It must be remembered that the nuts used in this study were produced in the crop year 1931, in which occurred the severest drought in the history of the pecan industry. Such cultural treatments as pruning or applications of nitrogen, which stimulated shoot growth with subsequent increased leaf area early in the spring before the beginning of the drought, have been shown to be of great importance in determining the ultimate size of the nuts and the degree to which they were filled.

The practice of pruning has been shown to have the effect of increasing the size of the nuts, or the percentage of nuts of the larger sizes. Furthermore, the nuts produced by pruned trees have been filled to a higher degree than those borne by similar unpruned trees. Pruning the trees removed a considerable portion of the tops and hence decreased the proportion of top to root. Although new shoot growth and the production of a large leaf area were greatly stimulated by pruning, the trees were able to withstand the conditions of the drought much better than unpruned trees, as evidenced by the larger and better filled nuts produced.

In some instances applications of nitrogenous fertilizers to pecan trees have been shown to increase the size of the nuts, or the percentage of nuts of the larger sizes, and the degree to which the nuts were filled. In other cases, the effects of nitrogenous fertilizers have been shown to decrease the average size and to lower the degree to which the nuts were filled. These effects are most probably the results of differences in the moisture supply that was available to the trees during the critical periods of growth or filling of the nuts. The effects of the fertilizer applications on the quality of the nuts have varied so widely among the different orchards that had not the nuts been sized and graded according to their degree of filling before analyses were made of their composition, erroneous conclusions possibly would have been drawn regarding the effects of fertilizers on nut composition.

The effect of the various fertilizer treatments on the composition of nuts of comparable size and degree of filling appears to be very slight, as indicated by the remarkable uniformity in the percentage composition of the nuts produced in any one orchard under the various treatments given. However, a comparison of the data on nuts of the same variety but from different orchards, as the Stuart nuts from orchards 1 and 2, indicates that the foregoing conclusion may not be entirely correct. Although no effect of treatment is evident in either orchard taken separately, the nuts from the more vigorous trees of orchard 1 had a consistently higher percentage content of protein than those from the weaker trees of orchard 2. The reason for this is not clear, although there is an indication that when trees are in poor growing condition, probably from a lack of nitrogen, a smaller amount of protein is stored in nuts of a certain

size and degree of filling than is normally the case. The fact that a wide range of fertilizer treatments was applied in orchard 2 with no apparent effect does not lessen the possibility that nut composition may be affected by a limited nitrogen supply, since these fertilizer treatments were first applied during the season in which the nuts used in this study were produced. Rainfall deficiency during the same growing season may have further minimized the effects of the treatments.

Further work will be necessary before final conclusions can be drawn on this phase of the study, although, as stated previously, the various combinations of fertilizer and pruning treatments appear to have had little direct effect on the percentage composition of nuts of comparable size and degree of filling, except possibly when the nitrogen supply had previously become a limiting factor, and only then after sufficient time had elapsed for the added nitrogen to be assimilated by the tree.

Size as a factor directly associated with a definite percentage composition of kernels has not been indicated by the data presented. Large nuts have neither a consistently higher nor a consistently lower percentage content of any of the constituents than do otherwise comparable small nuts, and what differences are present are usually small. The size of the nuts does determine to some extent the gross amounts of the constituents that can be stored, but apparently in no way affects the relative amounts stored.

The relationship between the degree of filling and the percentage composition of the kernels is outstanding. In all but a very few cases, out of the total number of comparisons possible between the data on samples comparable except as to the degree of filling, the better filled nuts had a higher percentage content of oil and a lower percentage content of protein, carbohydrates, water, and undetermined constituents than did the poorer filled nuts. As calculated by Student's method this relationship is significant by odds greater than 9,999:1. This also holds for the nuts 90 to 100 percent filled, the few available data of which are not presented here.

The fact that the chemical composition of the nuts is so directly associated with the degree of filling establishes the importance of the degree of filling as a requirement for market grades of pecans. Highest quality is directly associated with highest oil content and highest degree of filling. Thus, the determination of oil content and specific gravity, or degree of filling, may be a means of establishing the quality requirement of pecan grades.

The increase in percentage content of oil as the nuts become better filled is a factor that tends to lower the percentage content of the other constituents. To what extent this effect becomes apparent has been studied through various methods of analysis of the data. Ratios of a decrease in the other constituents to an increase in oil were found to be so variable and inconsistent as to warrant no definite conclusions without further study of the subject. The trend of these ratios, however, indicates that the decrease in percentage content of protein, carbohydrates, water, and undetermined constituents is not entirely dependent upon an increase in the percentage content of oil.

Methods of orchard management may markedly affect the degree of filling of the nuts and thus be associated with differences in com-

position. On the basis of the evidence obtained, treatments resulting in poorly filled nuts will result also in nuts low in oil content and relatively high in other constituents. On an average, treatments that give the best filled nuts will at the same time give nuts of the highest oil content.

SUMMARY

The practice of pruning pecan trees has been found to increase the percentage of large nuts. Pruned trees have produced nuts having a higher specific gravity and heavier kernels and filled to a higher degree than nuts of the same size borne by similar but unpruned trees.

In some instances applications of nitrogenous fertilizers have produced results similar to pruning; in other instances the fertilizer has reduced the percentage of large nuts. Likewise, under drought conditions, nitrogen applications have decreased the specific gravity, weight of kernel, and the degree to which the nuts were filled as compared with nuts produced by similar trees that received smaller amounts of nitrogen or none at all.

The percentage composition of pecan nuts of any particular variety is almost, if not entirely, dependent upon the degree of filling, size and cultural treatment having but little direct influence. A relatively high percentage content of oil and a relatively low percentage content of protein, carbohydrates, water, and undetermined constituents are associated with a high degree of filling in nuts of otherwise comparable samples. Thus oil content, or the degree of filling, becomes of great importance in the establishment of the requirements for market grades of pecans.

As great differences may be found between comparable nut samples of the same variety grown under different conditions as are to be found between comparable nut samples from different varieties. The evidence at hand indicates that these differences within a variety are caused by a limited supply of some essential element, probably nitrogen, rather than by a varied supply of such an element after the minimum requirements have been met.

The data obtained indicate that the results of the analyses of samples taken as an aliquot of the crop as a whole may lead to erroneous conclusions, since no account would thus be taken of the range of sizes and degree of filling of the nuts making up the samples. For this reason, the effect of a treatment on the nuts from experimental trees can probably be determined much more accurately by measuring the effect of the treatment on the size and the degree of filling of the nuts than by a chemical analysis of a representative sample.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1925. official and tentative methods of analysis. Compiled by the committee on editing methods of analysis . . . Ed. 2, 535 pp., illus. Washington, D.C.

(2) Fowler, E. D.
1931. a report of pecan fertilizers for the southeastern united states. Georgia-Florida Pecan Growers' Assoc. Proc. 25: 6–10.

(3) Jones, D. B.
1931. factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U.S. Dept. Agr. Circ. 193, 22 pp.

(4) LEWIS, R. D.
1932. DETERMINATION OF OIL CONTENT OF PECANS. NEW SULFURIC ACID DIGESTION METHOD. Indus. and Engin. Chem., Analyt. Ed. 4: 296–299.
(5) MARSHALL, H. L., and RUPRECHT, R. W.
1930. PECAN ANALYSES. Georgia-Florida Pecan Growers' Assoc. Proc. 24: 19–22.
(6) SHAFFER, P. A., and HARTMANN, A. F.
1921. THE IODOMETRIC DETERMINATION OF COPPER AND ITS USE IN SUGAR ANALYSIS. II. METHOD FOR THE DETERMINATION OF REDUCING SUGARS IN BLOOD, URINE, MILK, AND OTHER SOLUTIONS. Jour. Biol. Chem. 45: 365–390, illus.
(7) SKINNER, J. J.
1927. THE WORK OF THE BUREAU OF CHEMISTRY AND SOILS WITH PECANS. THE RELATION OF SOIL FERTILITY AND COMMERCIAL FERTILIZERS TO PECAN PRODUCTION. Natl. Pecan Growers' Assoc. Proc. 26: 52, 54, 56, 58–63.

INHERITANCE OF AWN DEVELOPMENT IN SORGHUMS[1]

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INTRODUCTION

Sorghum (*Sorghum vulgare* Pers.)[2] varieties may be divided into four classes on the basis of awn development, namely, strong-awned, weak-awned, tip-awned, and awnless (fig. 1.) In the strong-awned (fully awned) class are included the milos (except Wheatland milo), broomcorn, White durra, darso, shallu, most kaoliangs, and several varieties of sorgo, including Honey, Gooseneck, Freed, and most strains of Amber. Shantung kaoliang and some strains of hegari have homozygous weak awns, but with these exceptions plants having weak awns are heterozygous hybrids of strong-awned and tip-awned varieties. In the tip-awned class are included the feteritas, Grohoma (a feterita derivative), and Rice kafir. In the awnless class are the

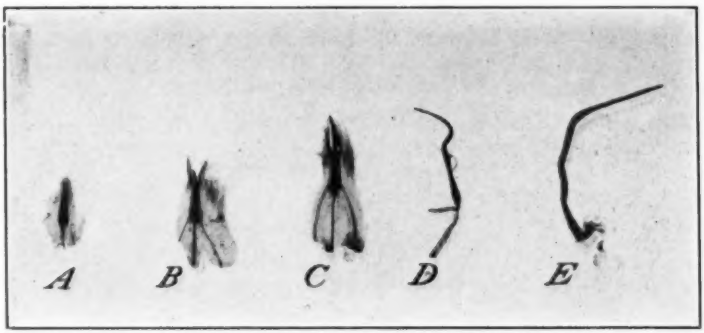

FIGURE 1.—Sorghum awns with lemmas or portions of lemmas attached showing awn classes: *A*, Awnless; *B*, awnless (F_1 hybrid between classes *A* and *C*); *C*, tip awn; *D*, weak awn (F_1 hybrid between *C* and *E*); *E*, strong awn. ×3.

kafirs (except Rice kafir) and many sorgos such as Sumac, Orange, Sapling, Atlas, and Sourless. The base of the awn is attached where the three nerves join, about the middle of the very thin, fragile lemma (fig. 1). In the awnless varieties there is merely a short-pointed rib that does not extend to the apex of the lemma. In the tip-awned varieties the point is extended slightly beyond the apex of the lemma, but the panicle appears awnless because the awns do not extend beyond the glumes. Strong awns extend considerably beyond the lemma and glumes, are usually half an inch or more in length, and in many varieties are twisted and geniculate. Weak awns extend beyond the glumes less than the strong awns and are somewhat more than half as long.

[1] Received for publication May 29, 1934; issued October 1934.
[2] Synonyms: *Holcus sorghum* L. and *Andropogon sorghum* Brot.

The awn is not known to have any protective or physiologic value to the sorghum plant. Awnless and tip-awned varieties are somewhat more agreeable to man and animals than strong-awned types, although the awn of sorghums is much less irritating than that of barley or wheat. A knowledge of the inheritance of awn development is of value largely in facilitating sorghum breeding and the inspection and purification of pure seed supplies.

PREVIOUS INVESTIGATIONS

Ramanthan [3] reported a segregation of 3 long-awned to 1 short-awned plant in sorghums. Vinall and Cron [4] described the F_1 plant of a cross between Dwarf milo and feterita as unawned and reported an F_2 segregation of 3 unawned plants to 1 awned plant. This is not in agreement with the results obtained on this and similar crosses by the writers. Crosses observed by the writers between milo (strong-awned) and feterita (tip-awned) have been weak-awned in the F_1 generation and have segregated for strong-awned, weak-awned, and tip-awned plants in F_2.

Parker [5] reported a segregation of awnless to awned plants approaching a 3:1 ratio in a cross between Kansas Orange sorgo (awnless) and Dwarf Yellow milo (awned).

MATERIALS AND METHODS

The varieties included in the crosses studied show the following awn-development characters:

Awnless	*C.I. no.*[6]	*Strong-awned*	*C.I. no*
Early Red kafir	866	Dwarf Yellow milo	332
Pink kafir	432	Leoti Red sorgo	---
Sunrise kafir	472	Dwarf White milo	627
Dawn kafir	340	White durra	81
Dwarf hegari	620	Blackhull kaoliang	310
Tip-awned		Dwarf Freed	971
Standard feterita	182	Dwarf White durra	---

Some strains of Dwarf hegari possess very short or weak awns that are almost entirely deciduous at maturity, but the one used in the cross with Leoti Red sorgo (table 1), grown in the F_2 generation at Woodward, Okla., in 1930, was awnless.

Data are presented for crosses involving all three possible combinations with the three awn types. The crosses were made by the senior author at the Southern Great Plains Field Station, Woodward, Okla.; by the second author at the Fort Hays Branch Station, Hays, Kans.; or by the junior author at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C. The F_1 and later generations were grown either at Woodward or Hays or at both places. The crossing was done either by emasculation, bagging, and subsequent pollination, or by bagging together heads of two varieties, growing the progenies of the two heads, and then identifying the F_1 hybrid plants by their hybrid vigor or other characters. None of the crosses was

[3] RAMANTHAN, V. SOME OBSERVATIONS ON MENDELIAN CHARACTERS IN SORGHUM. Jour. Madras Agr. Students' Union 12: 1–17, illus. 1924.

[4] VINALL, H. N., and CRON, A. B. IMPROVEMENT OF SORGHUMS BY HYBRIDIZATION. Jour. Heredity 12: 435–443, illus. 1921.

[5] HAYES, H. K., and GARBER, R. J. BREEDING CROP PLANTS. Ed. 2, 438 pp., illus. (See p. 247.) New York. 1927.

[6] C.I. denotes accession number of the Division of Cereal Crops and Diseases.

made specifically to study awn inheritance alone. The F_2 populations were nearly all grown from bagged heads and many of the F_3 lines were grown from bagged F_2 heads. Counts in segregating F_3 lines were confined to the progenies of selfed F_2 plants.

The notes on awn character were made either before or after maturity, but the identification of awned plants is easiest at the heading stage owing to the tendency of the awns to be deciduous later. It is necessary to open the glumes in order to distinguish between awnless and tip-awned plants.

RESULTS OBTAINED

Numerous crosses between two strong-awned varieties have produced only strong-awned segregates when protected from contamination by other varieties. Likewise, no cross between two awnless varieties or between an awnless and a tip-awned variety has produced any strong-awned segregates. Results are shown here for nine crosses in which the parental varieties were of different awn character.

AWNLESS VERSUS STRONG AWN

In all crosses between awnless and strong-awned varieties the F_1 plants have been awnless and the dominance nearly complete. The awn point of the hybrid plants was only slightly longer than that of the awnless parent and in all cases resembled that shown in figure 1, *B*. The segregation in the F_2 generation of five crosses is shown in table 1, and the behavior of one of the crosses in the F_3 generation

TABLE 1.—*Segregation in the F_2 generation of awn development in crosses between awnless and strong-awned sorghums*

Cross, station, and year grown	Awn class	Plants observed	Plants calculated (3:1 ratio)	Deviation
		Number	*Number*	*Number*
Early Red kafir×Dwarf Yellow milo (Woodward, Okla., 1929).	Awnless	500	495.75	4.25±7.51
	Strong-awned	161	165.25	
	Total	661		
Early Red kafir×Dwarf Yellow milo (Hays, Kans., 1929).	Awnless	249	240	9±5.22
	Strong-awned	71	80	
	Total	320		
Dwarf hegari×Leoti Red sorgo (Woodward, Okla., 1930).	Awnless	897	894.75	2.25±10.07
	Strong-awned	296	298.25	
	Total	1,193		
Dwarf White milo×Pink kafir (Hays, Kans., 1930)	Awnless	188	199.5	11.5±4.76
	Strong-awned	78	66.5	
	Total	266		
White durra×Sunrise kafir (Woodward, Okla., 1931)	Awnless	148	150	2.0±4.13
	Strong-awned	52	50	
	Total	200		
Sunrise kafir×Blackhull kaoliang (Woodward, Okla., 1931).	Awnless	335	337.5	2.5±6.20
	Strong-awned	115	112.5	
	Total	450		
All 5 crosses	Awnless	2,317	2,317.5	1.5±16.24
	Strong-awned	773	772.5	
	Total	3,090		

in table 2. The segregation in both generations is in all cases in remarkably close agreement with the calculated ratio of 3 awnless plants to 1 strong-awned plant. The progenies of 18 selected F_2 plants of the Early Red kafir × Dwarf Yellow milo cross were grown in the F_3 generation at Woodward, Okla., in 1930. Twelve of the fifteen progenies from the awnless F_2 plants segregated. This was slightly but not significantly more than the 10 to be expected on the basis of random assortment. It seems logical to conclude that the awnless character in sorghums differs from the strong-awned by a single dominant factor. The presence of the dominant awnless factor, even in a heterozygous condition, almost completely inhibits the extension of awn points.

TABLE 2.—*Awn development in the F_3 generation of a cross between an awnless variety (Early Red kafir) and a strong-awned variety (Dwarf Yellow milo) at Woodward, Okla., 1930*

Awn class	Rows	Plants in 3 segregating rows		Deviation
		Observed	Calculated (3:1 ratio)	
	Number	*Number*	*Number*	*Number*
Awnless	3	392	389.25	2.75±6.65
Strong-awned	3	127	129.75	
Segregating	12			
Total	18	519		

TIP AWN VERSUS STRONG AWN

The glumes of F_1 plants of crosses between the tip-awned feterita and sorghums with strong awns are described here as weak-awned (fig. 1, *D*). The weak awns are only about half as long as the strong awns and usually are not intensively geniculate or twisted. On the basis of the presence or absence of observable awns, the awn may be regarded as a partially dominant character in crosses between tip-awned and strong-awned varieties. The F_2 segregation in two crosses of Standard feterita with Dwarf Freed and Dwarf White durra is shown in table 3. In both crosses the plants segregated for strong awn, weak awn, and tip awn in numbers closely approximating a calculated 1:2:1 ratio. In the F_3 generation (table 4) the segregating progenies again showed good agreement with the calculated 1:2:1 ratio. At Hays, Kans., the strong- and weak-awned plants were grouped together for convenience; nevertheless the segregations of both the rows and plants agreed well with the single-factor basis suggested above.

The number of heterozygous lines and true-breeding strong-awned and tip-awned F_3 progenies of both crosses grown at Woodward were well within expected limits.

TABLE 3.—*Segregation in the F_2 generation for awn development in crosses between strong-awned and tip-awned sorghum varieties, 1929*

Cross and station	Awn class	Plants observed	Plants calculated (1:2:1 ratio)	χ^2	P
		Number	*Number*		
Feterita×Dwarf Freed (Woodward, Okla.)	Strong-awned	100	99.5	0.171	0.933
	Weak-awned	202	199.0		
	Tip-awned	96	99.5		
	Total	398			
Feterita×Dwarf Freed (Hays, Kans.)	Strong-awned	122	124.5	.972	.618
	Weak-awned	242	249.0		
	Tip-awned	134	124.5		
	Total	498			
Feterita×Dwarf White durra (Woodward, Okla.)	Strong-awned	76	84.5	1.527	.481
	Weak-awned	170	169.0		
	Tip-awned	92	84.5		
	Total	338			

TABLE 4.—*Behavior of awn development in the F_3 generation of crosses between strong-awned and tip-awned sorghums, 1930*

Cross and station	Awn class	Rows	Plants in segregating rows		χ^2	P
			Observed	Calculated		
		Number	*Number*	*Number*		
Feterita×Dwarf Freed (Woodward, Okla.)	Strong-awned	10	409	386	3.280	0.199
	Weak-awned	[1] 7	776	772		
	Tip-awned	7	359	386		
	Total	24	1,544			
Feterita×Dwarf Freed (Hays, Kans.)	Strong-awned and weak-awned.	[2] 151	[3] 567	562.5		
	Tip-awned	47	[3] 183	187.5		
	Total	198	750			
Feterita×Dwarf White durra (Woodward, Okla.).	Strong-awned	2	220	237.25	2.184	.341
	Weak-awned	[1] 6	477	474.50		
	Tip-awned	6	252	237.25		
	Total	14	949			

[1] Segregating (heterozygous).
[2] Fully awned or segregating. Deviation from 3:1 ratio 2.5±4.1.
[3] Deviation from 3:1 ratio 4.5±8.0.

In crosses of sorghums having the type of awnedness described as tip-awned with strong-awned varieties, the awned character apparently is inherited as a simple partial dominant.

AWNLESS VERSUS TIP AWN

In crosses between a kafir (awnless) and feterita (tip-awned) the F_1 plants have been almost as awnless as the kafir parent (fig. 1, *B*). In the F_2 generation the plants segregate for the awnless and tip-awn characters. The classification of the F_2 hybrid plants of two such crosses is shown in table 5. The determination of the awn character

is more difficult in these two crosses than in those in which the strong-awn character is involved. The results obtained, however, show a close agreement with a calculated 3:1 ratio for awnless and tip-awned plants. This indicates a single-factor difference, with the awnless character almost completely dominant.

TABLE 5.—*Segregation in the F_2 generation of awn development in crosses between awnless and tip-awned sorghums, 1930*

Cross and station	Awn class	Plants observed	Plants calculated (3:1 ratio)	Deviation
		Number	*Number*	*Number*
Sunrise kafir×Standard feterita (Woodward, Okla.)	Awnless	125	129	4.00±3.83
	Tip awned	47	43	
	Total	172		
Dawn kafir×Standard feterita (Hays, Kans.)	Awnless	363	363.75	.75±6.43
	Tip awned	122	121.25	
	Total	485		

DISCUSSION

On the basis of the data just presented, it is concluded that awnlessness is determined by a single factor almost completely dominant to both the strong-awn and tip-awn factors. The strong-awn character also is controlled by a single factor that is partially dominant to the tip-awn factor but recessive to the awnless factor. True-breeding F_3 progenies resembled one or the other of the parental varieties. From these facts it would seem that the inheritance of awn development is best explained on the basis of multiple allelomorphs. Other possible interpretations are not entirely precluded, but the simpler explanation is suggested of three multiple allelomorphic factors, namely, AA (awnless), aa (strong awn), and a^ta^t (tip awn), as determiners for awn development in sorghums.

SUMMARY

Sorghum varieties may be divided into four classes for awn development—strong-awned, tip-awned, weak-awned, and awnless. Data on the results from 9 crosses between 3 of these different awn classes, namely, strong-awned, tip-awned, and awnless, are presented. The awnless character is inherited as a simple dominant to both the strong-awn and tip-awn characters. The strong-awn character is inherited as a simple but partial dominant to the tip-awn character. The inheritance of awn development apparently is best explained by the assumption of three pairs of multiple allelomorphic factors, namely, AA (awnless), aa (strong awn), and a^ta^t (tip awn).

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